

Validation of Measurements of Bone Density, and
the Roles of Creatine and Exercise in
Musculoskeletal Health in Women

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For Simon

“Suck it up, Princess”

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List of Abbreviations

ADP	Adenosine Diphosphate
AGHD	Adult-onset Growth Hormone Deficiency
ALP	Alkaline Phosphatase
ATP	Adenosine Triphosphate
BIA	Bioelectrical Impedance Analysis
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
CK	Creatine Kinase
DMS	Dexamethasone
DXA	Dual Energy X-ray Absorptiometry
ELISA	Enzyme Linked Immunoabsorbent Assay
FFM	Fat-Free Mass
FM	Fat Mass
GAA	Guanadinoacetate
GH	Growth Hormone
GPA	β -Guanidinopropionic Acid
HRT	Hormone Replacement Therapy
IGF-1	Insulin-like Growth Factor 1
iPAQ	International Physical Activity Questionnaire

ISCD	International Society of Clinical Densitometry
LoA	Limits of Agreement
LSC	Least Significant Change
NTX	N-terminal teleopeptides
Olc	Osteocalcin
OPG	Osteoprotegerin
PCr	Phosphocreatine
Pi	Inorganic Phosphate
PTH	Parathyroid Hormone
RANK	Receptor Activator for Nuclear Factor K
RANKL	RANK Ligand
RDA	Recommended Dietary Allowance
RMS	Root Mean Square
RoI	Region of Interest
SHBG	Sex Hormone Binding Globulin
TH	Thyroid hormone
TSH	Thyroid Stimulating Hormone
WHO	World Health Organisation

Thesis Abstract

Osteoporosis is an increasing concern in today's ageing population. As individuals age, decreasing bone density, muscle mass, and muscle function contribute to low levels of independence, mobility, and quality of life. Therefore, there is an increasing need for interventions to counteract the degradation of bone and muscle. The present thesis sets out to investigate the effects of creatine supplementation, and the augmentation of exercise by creatine supplementation, on musculoskeletal health in adult women.

The studies in the present thesis begin in section 1 by establishing the reliability of the measurement techniques used, and the validity of the measurements of bone density and body composition during periods of oral supplementation with creatine monohydrate. Experimental studies in section 2 then begin by investigating the baseline relationship between physical activity, creatine intake, and musculoskeletal health through a cohort study. Finally, intervention studies investigate the long-term effects of creatine supplementation on musculoskeletal health, and the effects of 12 weeks' high-intensity exercise augmented by creatine supplementation on musculoskeletal health.

The findings of the present thesis demonstrate a positive relationship between muscle mass and bone density, and between muscle strength and bone density, in adult women. There does not appear to be a significant effect of creatine supplementation on bone density, although there is a significant increase in muscle strength following creatine supplementation. There is no significant effect of exercise on BMD over 12 weeks' intervention, but there is a significant positive effect of exercise on muscle strength, and on body composition, as seen in increased fat-free mass and decreased fat mass. The effects of exercise on musculoskeletal health were not augmented through creatine supplementation.

In conclusion, there was no significant effect of creatine supplementation on any markers of musculoskeletal health apart from muscle strength. Exercise has a significant beneficial effect on musculoskeletal health, but these effects are not augmented by creatine supplementation in this population. Future research should investigate the effects of higher creatine supplement doses, and longer exercise durations. Future studies should also focus on improving overall musculoskeletal health through improved muscle mass and strength, and the relationship between bone and

muscle health, as well as on the possible preferential uptake of creatine into muscle rather than bone tissue.

1 Introduction

In an ageing population, diminishing musculoskeletal health is an increasing concern. Osteoporosis, a bone degenerative disease characterised by low bone mineral density (BMD), affects 1 in 2 women over the age of 50 (van Staa et al., 2001), with the likelihood increasing after the menopause. Osteoporosis leads to an increased risk of suffering a fracture. In the UK, approximately £3,496 million was spent on fracture treatment and care in 2010, which is estimated to increase to £5,465 million by 2025 (Svedbom et al., 2013). Osteoporosis is associated with decreased quality of life, increased incidence of secondary fractures, and depression (Gold, 2001, Tosteson et al., 2001). In addition, osteoporotic fractures are associated with increased morbidity and mortality rates (Cooper et al., 1993, Keene et al., 1993, Leibson et al., 2002). Achieving greater bone mass early in life reduces the likelihood of developing osteoporosis later in life (Hansen et al., 1991, Hui et al., 1989).

There are currently a number of recognised treatments for osteoporosis, although these are generally associated with attenuation of further bone loss once osteoporosis has already been diagnosed, rather than with prevention of the disease onset. Treatments currently used for osteoporosis include nutritional calcium, vitamin D, oral or intravenous bisphosphonates, strontium ranelate and raloxifene, but all of these treatments are associated with pharmacological side-effects. Bisphosphonates are associated with dysphagia and stomach pain, strontium ranelate with diarrhoea and nausea, and raloxifene with increased risk of blood clots (Cranney and Adachi, 2005, Makins and Ballinger, 2003, Meunier et al., 2009). Although hormone replacement therapy has been shown to maintain bone density, it is not recommended for treating osteoporosis as it increases the risk of developing breast, ovarian, and endometrial cancer more than it lowers the risk of osteoporosis (Beral, 2003, Rossouw et al., 2002). Oral supplementation of calcium, or calcium with vitamin D, can slow bone loss and reduce fracture risk (Avenell et al., 2014, Tang et al., 2007), and is not associated with any severe adverse effects (Avenell et al., 2014). There is some discrepancy in the literature regarding the efficacy of calcium or calcium/vitamin D supplementation at decreasing fracture risk (Reid et al., 2006, Reid et al., 2008), particularly in individuals with sufficient dietary calcium intake (Dawson-Hughes et al., 1997, Orwoll et al., 1990, Shin and Kim, 2015). In addition, high compliance is necessary; supplementation with

calcium and vitamin D requires at least 80% compliance to be effective (Sunyecz, 2008). Vitamin D is essential in the body for the correct absorption of calcium; vitamin D intake of $15\mu\text{g}\cdot\text{day}^{-1}$ is recommended for optimal health, however supplementation with vitamin D alone does not reduce fracture risk in older adults, and excessive vitamin D supplementation above levels necessary for healthy growth can lead to hypercalcemia (Avenell et al., 2014).

Creatine is a naturally occurring compound, which can be obtained through the diet in foods such as red meat and fish, and can be synthesised by the liver and kidneys. Creatine is important in the body's energy pathway; supplementation with creatine allows more work with a high energy demand to be completed before fatigue occurs. Creatine is frequently used as an ergogenic aid in sporting contexts for increasing muscle gains, however in recent years it has been shown to have therapeutic properties in a number of disease states (Gualano et al., 2010). Creatine is used in bone growth, and *in vitro* studies have demonstrated that creatine supplementation results in increased activity of cells involved in bone growth (Gerber et al., 2005). Creatine supplementation has been shown to have the potential to improve bone health in ageing women (Gualano et al., 2010, Gualano et al., 2016), although this has not been conclusively shown. It has been shown to be safe for consumption and supplementation in humans (Juhn and Tarnopolsky, 1998, Poortmans and Francaux, 2000).

Physical activity during childhood and adolescence, defined as any movement of skeletal muscles which requires energy expenditure including activities of daily living such as walking and gardening, can positively influence bone mass accrual, and can attenuate bone loss in later life (Forwood et al., 2006, Janz et al., 2004, Khan et al., 2000, Linden et al., 2006). Given that increased pre-menopausal bone mass leads to a reduced risk of osteoporosis development, physical activity in adulthood may slow the onset of osteoporosis (Kelley et al., 2013, Vainionpaa et al., 2005, Zhao et al., 2014). Regular participation in high impact physical activity are associated with greater bone mass (Vainionpaa et al., 2005, Zhao et al., 2014). Although the terms 'physical activity' and 'exercise' are sometimes used interchangeably, exercise is defined as any physical activity which is planned, structured, and purposeful. Different exercise modes have been associated with varying effects on the skeleton, due to the varied loading that the

skeleton undergoes. Exercise involving high impact and high loads has been shown to be effective at promoting bone adaptations (Guadalupe-Grau et al., 2009).

Physical activity can increase the body's lean mass, and decrease fat mass. Muscle mass is positively associated with bone mass, either due to a direct relationship arising from the effect of muscle on bone, or an indirect relationship arising from other external factors such as physical activity levels or diet (Daly et al., 2004, Lang, 2011). Creatine supplementation increases the body's capacity to perform high-energy work, increasing the body's adaptation to exercise demands (Bemben and Lamont, 2005). Muscle strength describes the ability of a muscle to exert force in a single contraction; creatine supplementation results in increased muscle size and strength, and when used in combination with exercise results in greater increases in lean mass than exercise alone (Kreider, 2003, Rawson and Volek, 2003).

The high incidence of osteoporotic fracture in the general population, and the associated economic cost, show the importance of investigating strategies to decrease risk factors for developing osteoporosis. It is therefore increasingly important that studies investigate methods of increasing bone mass in premenopausal women, as well as decreasing bone loss in postmenopausal women, in order to find strategies which aim at prevention, rather than at treatment.

2 Review of Literature

This literature review aims to discuss these factors in depth in order to arrive at a better understanding of bone growth; in doing so, it will provide the underpinning of the rest of the thesis.

2.1 Bone Biology

Large amounts of research have been conducted on the growth and histology of bones. Understanding how bones grow and what interventions may benefit bone growth may lead to improved health in an ageing population. Bones are important in the human body for a variety of reasons. They are important for structure and support of the body as well as protection, and are also important for storing minerals. Bone is composed of extracellular bone matrix and bone cells; osteoblasts, osteocytes, and osteoclasts. The composition of the bone matrix is responsible for the characteristics of the bone; different components of the matrix provide different properties. A number of genetic and environmental factors influence bone health throughout life (Mikkola et al., 2009). The main factors which affect bone health are hormones, diet, and physical activity.

2.1.1 Bone Histology

In mature adult bone, organic material accounts for approximately 35% of the mass of the bone matrix, and inorganic material for the remaining 65%. The organic material is mostly composed of collagens and proteoglycans; collagen provides bones with flexible strength, and proteoglycans provide strength under compression. The inorganic material in the bone is primarily a calcium phosphate, in the form of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$).

Osteoblasts are the bone cells responsible for producing collagen and proteoglycans, through the Golgi apparatus. The osteoblasts form small organelles called vesicles, which accumulate calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions. These vesicles bud from the osteoblasts, and the contents form crystals of hydroxyapatite. In this way, mineralised bone matrix is formed. Formation of new bone by osteoblasts is known as ossification, or osteogenesis. Elongated cell processes from the osteoblasts connect to other cell processes on other osteoblasts; this forms an extracellular bony matrix, which surrounds the cells and the processes.

Osteoblasts which are surrounded by the cell matrix are called osteocytes; although these bone cells are relatively inactive, they play an important role in maintaining the bone matrix by providing a structure for the laying down of new bone. There are spaces in the bone matrix, lacunae, which are occupied by osteocytes. The spaces occupied by the osteocyte cell processes are known as canaliculi (see Figure 2.1).

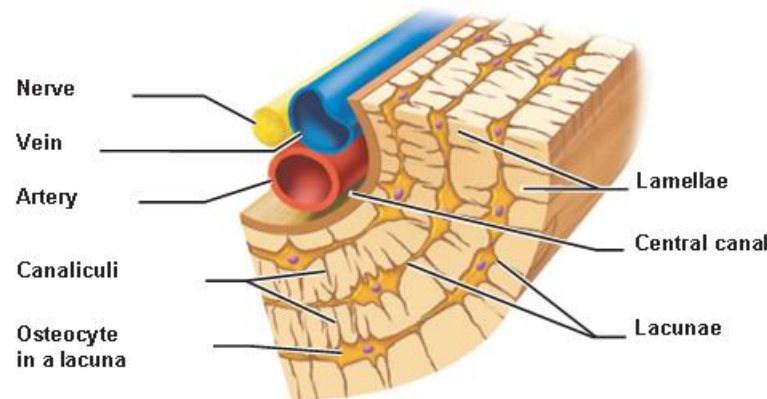


Figure 2.1: Structure of cortical bone (Source: Human Anatomy & Physiology, 8th edition, p. 181, by Marieb, E. and Hoehn, K., 2010, Pearson Education Inc., San Francisco)

These canaliculi provide a "mould", around which the bone matrix is formed. The bone cell processes have contact with one another via the canaliculi, enabling nutrients to pass from cell to cell through the canaliculi, rather than diffusing through the mineralised matrix as happens in the cartilage. Osteoblasts have a high energy demand, and so cellular metabolism and mitochondrial activity are both important in osteoblast function (Komarova et al., 2000).

While osteoblasts and osteocytes are responsible for bone growth, osteoclasts are responsible for bone resorption. These are large multinucleate cells, formed by the fusion of several macrophages, derived from hematopoietic stem cells (blood cells located in the red bone marrow and derived from the mesoderm) (Bar-Shavit, 2007). Osteoclastogenesis is indirectly stimulated by parathyroid hormone (PTH) (Boabaid et al., 2004). PTH receptors are present on osteoblasts, which in turn signal to osteoblast precursor cells, stimulating cell fusion, differentiation, and activation; this occurs via the RANK/RANKL pathway. Osteoclast precursors express a receptor known as receptor activator for nuclear factor K (RANK) (Karsenty and Wagner, 2002). Osteoblasts express RANK Ligand (RANKL), which binds to RANK; this activates

signalling pathways, promoting osteoclast differentiation (Boabaid et al., 2004); (Karsenty and Wagner, 2002).

It has been found that larger osteoclasts are present in diseases which present with excessive bone resorption (Jin et al., 2008). Osteoclasts have a "ruffled border", which creates a seal against the bone matrix. Hydrogen ions are then pumped across, producing an acid environment which causes the bone matrix to decalcify. Osteoclasts also secrete lysosomal enzymes, which allow the osteoclasts to digest some of the proteins from the bone matrix through endocytosis. Although the osteoclasts are the cells responsible for the resorption of bone, the process is assisted by the osteoblasts; the bone mineralised bone matrix is covered by a thin layer of unmineralised organic matrix. This unmineralised matrix is not easily broken down by the osteoclasts. Instead, the unmineralised matrix is broken down by osteoblasts, allowing the osteoclasts to come into contact with and breakdown the mineralised matrix underneath. It has been shown that a decrease in the number of active osteoclasts in otherwise healthy rats results in an increase in bone mineral density (BMD) (Yasuda et al., 1998).

2.1.2 Bone Growth

Bone growth in size occurs via appositional growth, which is the formation of new bone on the surface of cartilage or old bone. Until puberty, long bones have epiphyseal plates which separate the diaphysis from the epiphysis and are responsible for growth in length. After puberty, this plate has closed to form the epiphyseal line (see Figure 2.2). Long bones, and the spiny processes of bone such as the vertebrae, increase in length through growth at the epiphyseal plate. This happens through formation of new cartilage by interstitial growth, followed by appositional bone growth on the surface of the cartilage.

The epiphyseal plate contains 4 zones; growth happens at distinct phases in each zone. The zone nearest the epiphysis is the zone of resting cartilage; in this zone are randomly arranged chondrocytes. These chondrocytes do not divide rapidly. The next zone is the zone of proliferation; the chondrocytes divide and form columns, creating new cartilage through interstitial growth. The zone of hypertrophy contains chondrocytes which were produced in the zone of proliferation. These chondrocytes mature and enlarge. This means that the columns of chondrocytes have a maturation gradient, with the younger

cells, nearer the epiphysis, actively proliferating, and the older cells, nearer the diaphysis, undergoing hypertrophy. The final zone is the zone of calcification. This zone is a very thin layer containing a cartilage matrix mineralised with calcium carbonate. The hypertrophied chondrocytes die, and the blood vessels from the diaphysis grow into the area; the connecting tissues surrounding the blood vessels contain osteoblasts from the endosteum, which line up on the surface of the calcified cartilage, depositing bone matrix through appositional growth. This bone can then later be remodelled as part of the remodelling process (see 2.1.3). Throughout this process, although the length of the diaphysis increases, the thickness of the epiphyseal plate does not change, as the cartilage is built and calcified at either side of the plate at equal rates. During late stages of puberty, bone growth ceases; the epiphyseal plate closes, becoming the epiphyseal line. It has been demonstrated (Weise et al., 2001) that closure of the epiphyseal plate occurs when the proliferation potential of the chondrocytes in the growth plate ends. The ending of the proliferation potential of these chondrocytes is stimulated by the sex hormones, oestrogen and testosterone, although oestrogen has been shown to play a predominant role (Juul, 2001). The epiphyseal growth plates are also affected by levels of physical activity in early life; this will be discussed in greater depth in subsequent sections (see 2.1.7). This closure of the plate happens between the ages of approximately 12 and 25 years (Crowder and Austin, 2005, O'Connor et al., 2008, Sengupta, 2013).

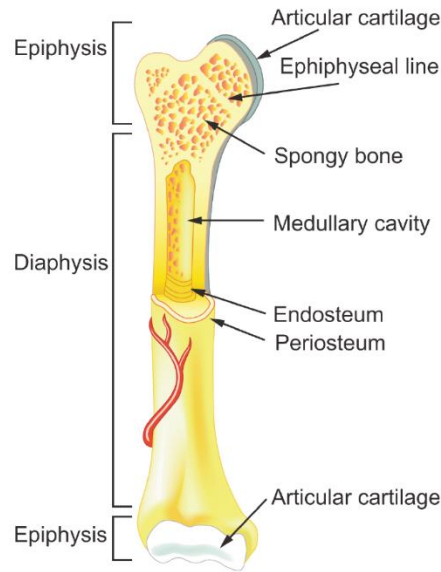


Figure 2.2: Structure of long adult bones; before puberty, the epiphyseal plate has not closed to become the epiphyseal line (Source: Principles of Anatomy and Physiology, 9th edition, p.161, by Tortora, G., and Garbowski, S., 2000, John Wiley & Sons, Inc., New York).

Bone growth of the long bones occurs not only via appositional growth, but also at the articular cartilage; this growth at the articular cartilage results in larger epiphyses (Lui et al., 2014). Bone growth at the articular cartilage also causes growth in short bones. Once the epiphyses reach full size, growth of the cartilage and the cartilage replacement by bone ceases (Rivas and Shapiro, 2002). However, articular cartilage does not become ossified.

Appositional bone growth is responsible both for an increase in the diameter of long bones, and an increase in the overall size of other bones (Clarke, 2008). Osteoprogenitor cells from the periosteum undergo mitotic division and differentiation to become osteoblasts, which can then deposit new bone matrix through appositional bone growth (Clarke, 2008). Trabecular bone grows through appositional bone growth; new bone matrix is added to the outer surface of the trabeculae. Appositional bone growth is also responsible for the growth of compact bone, through the formation of circumferential and concentric lamellae.

Continued growth of bone, at the epiphyseal plate and through articular and appositional growth, would result in large increases in bone mass. However, during appositional bone growth, osteoclasts are active on the endosteum, the lining of the medullary cavity in long bones (Clarke, 2008). Through the action of these osteoclasts,

the lumen increases in size; this allows the bone to grow in diameter but without a corresponding increase in the mass of the bone.

2.1.3 Bone Remodelling

Bone remodelling is an active process which occurs throughout life. It is tightly controlled, and carried out by osteoclasts and osteoblasts. Immature bone is characterised by having a seemingly random arrangement of fibres which are not oriented in the lines of stress (Weiner and Wagner, 1998). This bone has a high rate of turnover, and is very flexible and weak; one of the functions of the remodelling process is to convert this woven bone into lamellar bone, which is stronger and less flexible, with fibres organised and oriented in the lines of stress (Boyde, 1980). Bone remodelling is also responsible for bone growth, for bones adapting to the stresses they undergo, for changes in bone shape and size (growth and adaptation), for repair of the bone following injury or damage, and for calcium regulation in the blood (Hadjidakis and Androulakis, 2006).

Complementary activity of the osteoblasts and osteoclasts results in bone remodelling. As described, osteoclasts break down old or damaged bone, releasing stored calcium into the extracellular fluid. While osteoclasts are breaking down bone, osteoblasts act to build bone as described above, by laying down new bone matrix (Hadjidakis and Androulakis, 2006).

An osteon is the basic structural unit of cortical bone (see Figure 2.3), usually arranged parallel to the axis of stress (Koch, 1917, Singh et al., 1970). Osteons are constantly being removed and built, through osteoblast and osteoclast activity. During appositional bone growth (described above), osteoblasts beneath the periosteum secrete bone matrix (Bancroft et al., 2002). This new bone matrix forms ridges which follow the lines of the periosteal blood vessels. As these ridges grow, they meet, forming a tunnel through which the blood vessel passes. This new bone builds lamellae around the capillaries, creating new osteons, and increasing the diameter of the bone (Clarke, 2008). In existing osteons, osteoclasts enter the central canal through the blood vessels, and breakdown bone from the centre of the osteon. This creates a large channel through the bone. Osteoblasts follow the osteoclasts along this channel, laying new bone. In this way, bone is built up in layers within the channel, forming concentric lamellae. This

continues until the central channel, the Haversian canal, is not much wider than the central blood vessel. This creates a new osteon, which replaces the old (Buckwalter and Cooper, 1987). Old, partially resorbed osteons, in between new, are known as interstitial lamellae.

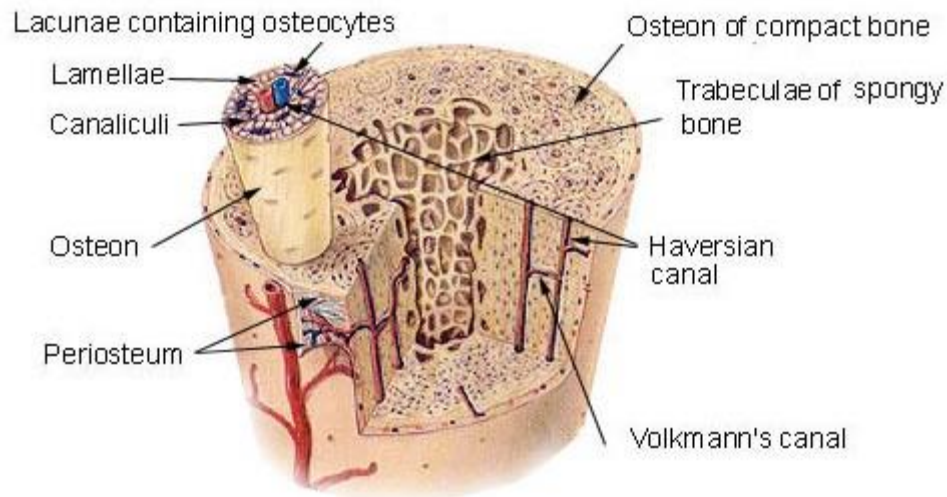


Figure 2.3: Structure of osteons within the cortical bone (Source: *Human Anatomy & Physiology*, 8th edition, p.181, by Marieb, E. and Hoehn, K., 2010, Pearson Education Inc., San Francisco).

Bone remodelling allows the bone to adapt to stress which it undergoes. Mechanical stress stimulates osteoblast and osteocyte activity, up regulating the expression of the genes associated with osteoblast activity. This leads to an increase in cell proliferation, and an increase in the laying down of new bone. Mechanical stress also suppresses osteoclast activity, leading to a decrease in bone resorption. In addition, mechanical stress promotes the secretion of osteoprotegerin (OPG) by the osteoblasts. OPG is a protein which acts to inhibit bone resorption by acting as a “decoy” receptor for RANKL, preventing RANKL from binding with RANK, and therefore inhibiting osteoclastogenesis (see 2.1.7).

Conversely, a decrease in mechanical stress leads to a decrease in osteoblast activity (Cooper et al., 1999). However, reduced stress on the bone due to paralysis or immobility does not affect osteoclast activity, meaning that osteoclast activity remains normal, and so there is a decrease in the laying down of new bone, but the rate of bone resorption remains normal. This leads to a decrease in bone density. These changes can begin to happen within 5 weeks of immobility (Berg et al., 2007). Prolonged

immobility also results in muscle atrophy, and hypercalcemia, high levels of calcium clearance. Hypercalcemia can set in within 3 weeks of immobility. It has been found that, within 12 weeks of immobility, there is an increase in the size of the periosteocytic lacunae, suggesting an increase in bone resorption (Minaire et al., 1974). It has been demonstrated that one week's immobility may result in bone loss at a rate as great as 2% (LeBlanc et al., 1987, Hansson et al., 1975, Hamdy et al., 1995).

2.1.4 Calcium Homeostasis

Calcium, in the form of calcium hydroxyapatite crystals, is one of the main mineral components of bone (Wang et al., 2006). The bones are the major site of storage of calcium in the body. Calcium is used in the body in a number of processes, including blood clotting, muscle contraction, and release of neurotransmitters, as well as in the bones and teeth (Hadjidakis and Androulakis, 2006). There is a high turnover of the calcium stores in the bone in order to maintain calcium levels in the blood with a narrow range; total blood calcium levels in a healthy adult range from $2.1\text{-}2.5\text{mmol}\cdot\text{L}^{-1}$, with ionised calcium levels in the range $1.3\text{-}1.5\text{mmol}\cdot\text{L}^{-1}$. Activity of the osteoblasts and osteoclasts regulate calcium homeostasis by allowing transfer of calcium into and out of the bone (Clarke, 2008, Hadjidakis and Androulakis, 2006). When osteoblasts and osteoclast activity are matched, the levels of calcium in the blood remain stable. If the levels of calcium in the blood are too low, osteoclast activity is increased, meaning that osteoclast activity exceeds osteoblast activity; more calcium is therefore released into the blood than is transferred into the bone, leading to an increased level of calcium in the blood. If the levels of calcium in the blood are too low, osteoclast activity is decreased. This means that osteoblast activity exceeds osteoclast activity, and so more calcium is transferred into the bones than into the blood, leading to a decrease in blood levels of calcium (Tai et al., 2015).

Calcium homeostasis is carefully maintained by hormones in the body. Parathyroid hormone (PTH), released from the parathyroid gland, is the major hormone responsible for regulation of the levels of calcium in the blood (Poole and Reeve, 2005). PTH operates to maintain calcium levels via a negative feedback loop; a decrease in blood calcium levels stimulates an increase in PTH release from the parathyroid gland (Potts and Gardella, 2007). This leads to an increase in osteoclast activity, and, as a consequence, an increase in blood calcium levels. Release of PTH also stimulates an

increase in vitamin D synthesis in the kidneys, leading to increased absorption of calcium from the small intestine and so an increase in the calcium levels in the blood. In addition to this, release of PTH causes an increase in the reabsorption of calcium from the urine in the kidneys, leading to a decrease in the amount of calcium lost in the urine (DeLuca et al., 1962). Conversely, an increase in blood calcium levels leads to a decrease in PTH release (Potts and Gardella, 2007). This decrease in PTH levels leads to a decrease in osteoclast activity (Attie, 1989), and so a decrease in the amount of calcium released from the bone, and consequently a decrease in blood calcium levels (Brown, 1982).

Tumours which secrete PTH can therefore lead to an increase in bone breakdown, and therefore a weakening of the bones (Adams, 1989, Philbrick et al., 1996). However, PTH does not directly cause an increase in osteoclast activity. Instead, an increase in PTH stimulates PTH receptors on the osteoblasts. This causes osteoblasts to release factors which stimulate osteoclast activity. This stimulation of osteoblasts by PTH causes the osteoblasts to release enzymes which break down the unmineralised matrix covering the bone, allowing the osteoclasts to reach the mineralised bone matrix beneath (McSheehy and Chambers, 1986).

Calcitonin, released from the thyroid gland, also plays a role in calcium homeostasis by causing a decrease in blood calcium levels (Friedman and Raisz, 1965); this happens in 4 ways. Calcitonin has a direct effect on osteoclasts, reducing both their number and activity (Singer et al., 1976). Osteoclasts exhibit a calcitonin receptor, which causes osteoclast activity to be inhibited by the secretion of calcitonin (Nicholson et al., 1986). This reduced osteoclast activity causes a decrease in the amount of calcium being released from the bones into the blood. Calcitonin secretion also stimulates the activity of osteoblasts (Farley et al., 1992), causing an increase in bone formation and a subsequent increase in the amount of calcium being taken up into the bone matrix. Increased secretion of calcitonin also results in inhibition of calcium absorption, both in the kidneys and in the intestines; this results in decreased levels of calcium in the blood, and increased levels of calcium being excreted in the urine (Friedman and Raisz, 1965, Olson et al., 1972).

2.1.5 Bone Mass Accrual, Consolidation, and Loss

Bone mass is often used as a marker of bone health. Throughout life, “normal” bone mass goes through 3 distinct phases (see Figure 2.4). These three distinct phases of bone mass are controlled and affected by both genetic and environmental factors (Baudoin et al., 2002, Jouanny et al., 1995, Smith et al., 1973). Genetic factors account for approximately 70% of an individuals’ bone health (Deng et al., 1999, Huang et al., 2004, Recker and Deng, 2002); the three main environmental factors are hormones, diet, and physical activity.

Bone growth happens from the foetal stage, and continues until the mid-twenties, when it reaches peak BMD; 99% of peak bone mass is reached by the age of 27 (Abrams, 2003, Schnatz et al., 2010, Specker et al., 2010). At this point bone growth ceases, and the maintenance, or consolidation, phase begins. For the next 20 years (approximately), there is overall no change in bone density; there is an equilibrium between osteoblast and osteoclast activity, with no significant net changes (Bielby et al., 2007). However, it has been demonstrated (Guadalupe-Grau et al., 2009) that adults who undergo high impact activities can exhibit small increases in bone mass through this period, although at a slower rate than in early life (see 2.2).

The third phase of bone mass is bone loss. In later adulthood, there is a change in the activity levels of osteoblasts and osteoclasts. In late adulthood, osteoclast activity exceeds osteoblast activity, leading to a decrease in bone mass. This decrease in bone mass is a loss of bone density; it is seen in increased size of the lacunae, and resorption of the collagen fibres around the lacunae. Trabecular bone in particular, because it has a higher rate of turnover than cortical bone, exhibits signs of thinning and breaking. It has even been observed that in some cases, granular bone crystals could be identified in osteoporotic bone (Shen et al., 2009). Because this decrease in bone mass is a loss of bone density, the bones become weaker; some of the bone matrix within the bone is lost, causing the bones to become less able to withstand forces. These bones are therefore more susceptible to breaking.

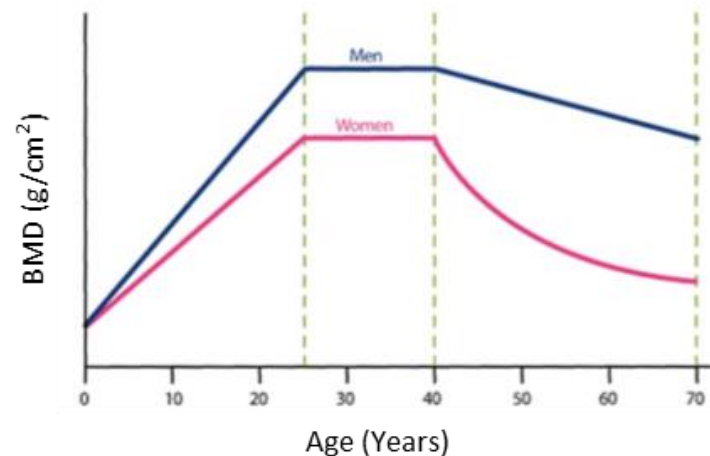


Figure 2.4: Bone mass throughout life

It has been shown that the bones of adult females are weaker relative to muscle mass than those of adult males; they do not build comparatively more cortical bone, but have a high proportion of trabecular bone. As a result of this, the bones are weaker (Jepsen et al., 2015). As they undergo the menopause, women experience a large decrease in oestrogen levels. This is accompanied by a rapid loss of trabecular bone (Seifert-Klauss et al., 2012); because osteoclasts act primarily on the surface of the bone, the higher surface area-to-volume ratio of trabecular bone compared to cortical bone means that trabecular bone loss occurs at a higher rate. This bone loss eventually leads to osteoporosis. In adults over the age of 50, osteoporosis is estimated to affect 1 in 2 women and 1 in 5 men (Kanis et al., 2000, Melton et al., 1998, van Staa et al., 2001). It is more common in women due both to the weaker bones throughout life, and to the large change in hormones which happens as a natural part of ageing, when women undergo the menopause.

2.1.6 Osteoporosis

A large decrease in bone density can lead to osteoporosis, a bone disorder which is characterised by having brittle, or porous bones (see Figure 2.5). In osteoporotic bone, the rate of bone resorption exceeds the rate of bone formation, leading to a net loss of bone mass. Osteoporotic bones are therefore weaker, and more susceptible to fracture. Primary osteoporosis is the onset of osteoporosis with age; it is particularly prevalent in postmenopausal women. Secondary osteoporosis refers to the onset of the disorder due to a previous clinical disorder such as hyperparathyroidism or anorexia nervosa. This

bone loss occurs particularly in the trabecular bone, which has a higher rate of turnover than cortical bone due to its larger surface-area-to-volume ratio; it is therefore more susceptible to the altered osteoclast-osteoblast activity ratio. Loss of BMD has been shown to be a risk factor for subsequent fracture in older men (Cawthon, 2013), and several studies have demonstrated the correlation between BMD and bone strength (Cheng, 1998, Dennison, 1999, Faulkner, 2000, Kanis et al., 1997).

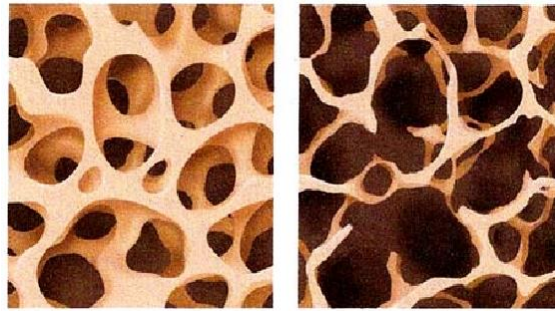


Figure 2.5: Healthy bone (left) and osteoporotic bone (right)

The clinical tool for diagnosing osteoporosis is dual energy X-ray absorptiometry (DXA). Osteoporosis is defined by the World Health Organisation (WHO) as having a DXA t-score of -2.5 or less, which is a BMD score more than 2.5 standard deviations below the mean of a young adult population's peak bone mass. The likelihood of osteoporosis onset increases with age. Apart from the impact of osteoporosis on healthcare systems, there is a significant relationship between BMD and quality of life (Paker et al., 2012). Osteopenia, a precursor to osteoporosis, results in significantly lower quality of life (Hakestad et al., 2014).

After the age of approximately 40, there is a decrease in bone mass because of the imbalance between bone resorption and formation. It is estimated that, over the age of 50, approximately 50% of women and 20% of men suffer from an osteoporotic fracture (Kanis et al., 2000, Melton et al., 1998, van Staa et al., 2001). Women can lose up to 50%, and men up to 25%, of the trabecular bone mass (Finkelstein et al., 2008, Hunter and Sambrook, 2000). The higher prevalence of bone loss in women than in men is explained by findings that the menopause has a greater effect on bone loss than individual age does (Nilas and Christiansen, 1987). During the menopause, the ovaries stop releasing eggs, and cease to function. The ovaries release large amounts of oestrogen, and so after the menopause there is a large decrease in the amount of

oestrogen in the body. Therefore, it can be concluded that the hormonal change that happens during the menopause has more of an effect on bone turnover than ageing alone.

Postmenopausal women suffering from osteopenic fracture have a reduced quality of life (Hakestad et al., 2014), and are the most at-risk group for osteoporosis, due to the sudden decrease in oestrogen which happens during the menopause (Khosla et al., 2012, Lanham-New, 2008, Sambrook et al., 1993). Oestrogen, due to its effect on PTH of decreasing the impact that PTH has on osteoclasts, helps to maintain BMD. The occurrence of the menopause therefore, with the decrease in oestrogen, results in a decrease in the bone mass of trabecular bone. When this happens in the lumbar spine it can lead to vertebral fractures, and can result in kyphosis of the upper back (or "widow's hump"). There are several conditions which can cause this decrease in oestrogen levels and subsequent decrease in BMD before the menopause occurs; premenopausal removal of the ovaries, over-exercising to the point of amenorrhoea, anorexia nervosa, and cigarette smoking all cause a decrease in the levels of oestrogen and so are risk factors for osteoporosis (Kelsey, 1989, Davies et al., 1990, Rigotti et al., 1991). Osteoporosis can also be caused by an overproduction of PTH (Burgess et al., 1999). This could be caused by a tumour, or by a parathyroid gland disorder.

Due to increasing life expectancy, the occurrence of osteoporosis in men is increasing (Szulc and Delmas, 2001, Drake et al., 2012). The decrease in testosterone levels as men age leads to a loss of bone tissue, and a subsequent decrease in bone mass. Osteoporosis is less common in men than in women; at peak bone mass, men have higher bone density than women. Also, testosterone does not decrease significantly until the approximate age of 65, and it has a slower rate of decline than oestrogen. Therefore, men have a higher peak bone density, and do not lose bone mass as quickly as women.

Insufficient calcium uptake, either from insufficient dietary calcium or from decreased calcium absorption by the small intestine, can lead to osteoporosis. This is because osteoclast activity, stimulated by the low levels of blood calcium, will break down bone mass to release calcium into the blood. As individuals age, there is a decrease in calcium absorption from the small intestine (see 2.1.4). Insufficient calcium, vitamin D, and vitamin C all lead to an increased risk of osteoporosis, as does the consumption of

any drugs which may affect either the rate of calcium uptake or the ability of the body to use calcium.

Exercise, or physical activity, causes bone loading through mechanical stress. Loads placed upon the bone result in microfractures in the bone matrix; the old damaged bone is broken down by osteoclasts, and replaced by osteoblasts. Osteocytes within the bone matrix are sensitive to fluid changes which occur during loading, and regulate osteoblast and osteoclast activity (see 2.1). This ability of bones to adapt to loads placed upon them means that bone formation occurs along lines of loading, strengthening the bone in the direction of the load. As individuals age, there is an imbalance in the activity of osteoclasts and osteoblasts, with the rate of bone resorption exceeding the rate of bone formation. However, physical activity can prevent bone loss by inhibiting bone resorption and by stimulating bone formation (Bergstrom et al., 2012, Smith et al., 1989a, Roghani et al., 2013). Low levels of exercise can lead to osteoporosis, as can disuse due to fractures or paralysis (Takata and Yasui, 2001).

2.1.6.1 *Treatment*

There are currently several forms of treatment for osteoporosis. These treatments reduce bone loss, and in some instances can increase bone formation. An increase in calcium and vitamin D intake can lead to an increase in calcium uptake, and a subsequent increase in bone formation (Dawson-Hughes et al., 1997). Calcium and supplementation can also result in increases in bone formation (Shea et al., 2004, Tang et al., 2007), however supplementation with both vitamin D and calcium is less effective in individuals who have sufficient dietary intake (Shin and Kim, 2015). Supplementation with both vitamin D and calcium requires at least 80% compliance for effectiveness (Sunyecz, 2008), but a long-term study investigating vitamin D and/or calcium supplementation found that only 54.5% of participants were taking the supplement after 24 months (Grant et al., 2005). Another treatment given for osteoporosis is calcitonin, which causes a decrease in osteoclast activity, and so a decrease in bone breakdown (see 2.1.4). Calcitonin can also result in a slight increase in bone mass, and can be prescribed to both men and women. Slow-releasing sodium fluoride in combination with calcium citrate can also increase bone mass, and it has been suggested (Kim and Ilich, 2011) that there may be a therapeutic effect on osteoporotic bone following supplementation with alpha-linoleic acid. Exercise can

also stimulate bone formation, leading to a decrease in bone loss. There is some suggestion that exercise could even increase bone mass if physical activity is sustained at a sufficient level (Hamilton et al., 2010).

Postmenopausal women diagnosed with osteoporosis can be given oestrogen replacement therapy (HRT). The increase of oestrogen levels results in a decrease in osteoclast activity (above), and so a decrease in bone loss. Although this treatment results in attenuation of loss of bone mass, it is not recommended for prevention of osteoporosis development due to the associated increased risk factors for breast, ovarian, and endometrial cancer (Beral, 2003, Rossouw et al., 2002).

Biphosphonates such as alendronate bind with hydroxyapatite, and decrease bone resorption by binding with hydroxyapatite crystals and so inhibiting breakdown of the hydroxyapatite crystals by osteoclasts (Russell et al., 1970). Alendronate treatment can increase bone mass and lead to a decrease in fracture rate more effectively than calcitonin. It has been shown that the use of antiresorptive drugs can result in increases in BMD, and also a reduction in the incidence of fracture (Cummings, 2002). Alendronate therapy has been shown to be more effective than hormone replacement therapy at increasing bone mass in older women; combination therapy of both alendronate and hormone replacement was more effective than either therapy alone (Greenspan et al., 2003). However, biphosphonates which are bound to bone have been shown to have an effect on non-bone tissues, inhibiting their growth (Cornish et al., 2011). Caution should therefore be used when recommending the use of biphosphonates such as alendronate.

Early diagnosis of osteoporosis, or identification of a low BMD pre-osteoporotic state (osteopenia), means that preventative treatments can be used. Although it has been stated that osteopenia should not be used for diagnostic purposes, but rather should be used only for epidemiological description (Kanis et al., 2013) it has been suggested that identification of women with high bone loss during the early postmenopausal phase, would allow early introduction of therapeutic interventions (Christiansen, 1987). However, this solution still aims to cure, rather than at prevention. Higher peak bone density prior to the menopause means that even an identical absolute rate of bone loss does not lead to osteoporosis as early in life; coupled with an attenuation of bone loss

after the menopause, it may be possible to significantly delay the onset of osteoporosis (Hansen et al., 1991).

2.1.7 Hormonal regulation of bone health

Hormones play an important role in the growth and maintenance of adequate bone mass. Several hormones affect bone growth and the bone remodelling process. These hormones include growth hormone, parathyroid hormone, oestrogen, calcitonin, thyroid stimulating hormone, vitamin D, OPG, leptin, and serotonin. Bone cells also produce hormones; osteoblasts produce fibroblast growth factor 23, and osteocalcin.

The most common contributor to osteoporosis is oestrogen deficiency, which normally occurs as a result of women undergoing the menopause. However, other causes of osteoporosis can include primary hyperparathyroidism, hyperthyroidism, excess cortisol production, and low testosterone levels, among other endocrine disorders. Throughout life, hormones are responsible for regulating bone growth and bone resorption, thereby regulating overall changes in bone mass.

2.1.7.1 *Hormonal regulation of bone mass accrual*

The anterior pituitary gland releases growth hormone, which increases the growth of body tissues in general, including bone growth through interstitial cartilage growth as well as appositional bone growth. It has been demonstrated (Mavalli et al., 2010) that growth hormone (GH) regulates skeletal muscle development in mice, and furthermore that GH affects both bone size and bone mass (DiGirolamo et al., 2007) as well as stimulating production of insulin-like growth factor 1 (IGF-1) in the liver and the bone, which has an additional stimulatory effect on osteoblast activity. It has been found that a deficiency of GH prevents long bone maturation, but that this disruption is normalised following treatment with GH supplementation (Even et al., 2014). However, it has also been demonstrated that, when bone size is corrected for bone mineral content (BMC), GH levels were not associated with bone mass, but with bone size and geometry (Gahlot et al., 2012). Correct levels of thyroid hormones are necessary for the normal growth of all tissues; deficiency of thyroid hormones in childhood can lead to impaired growth, and so shortness in adult life, while increased levels of PTH is associated with an increase in bone turnover, poor bone quality, and decreased bone strength (Radetti et al., 2014).

Androgenic hormones, oestrogen and testosterone, are also responsible for normal growth of the bone. Both of these hormones initially stimulate bone growth by stimulating osteoblast synthesis, and also by decreasing OPG and RANKL production by the osteoblasts, therefore inhibiting osteoclast activity; this also causes osteoclast apoptosis. There is a large increase in the levels of androgenic hormones at the onset of puberty, which explains the accompanying growth spurt. Both of these hormones also stimulate the ossification or "closure" of the epiphyseal plate, therefore leading to the cessation of growth; it has been suggested that this is through stimulating accelerated growth plate senescence (Weise et al., 2001) and so quickening the proliferative exhaustion of the chondrocytes. Oestrogen causes closure of the epiphyseal plate sooner than testosterone does, which is why on average females stop growing sooner than males. It has been shown that oestrogen level is positively correlated with bone maturation and BMD. Testosterone has been shown to be associated with lean mass and with bone size (Vandewalle et al., 2014). It has also been found that serum oestrogen regulates bone turnover and bone mass in both pubertal males and females; oestrogen indirectly regulates PTH. Increased levels of PTH stimulate bone turnover, and also cause an earlier onset of puberty (Csakvary et al., 2013).

2.1.7.2 Hormonal regulation of bone consolidation

The hormones which play the largest roles in maintenance of bone density are thyroid hormone (TH), GH, PTH, and androgenic hormones. Each of these plays a different role in maintenance of bone mass, and they work in conjunction to ensure healthy bone turnover.

Thyroid hormone is necessary in the body for skeletal growth. In children, hypothyroidism results in delayed growth, and hyperthyroidism leads to accelerated skeletal maturation. In adults, TH regulates bone turnover and therefore BMD. Hypothyroidism and hyperthyroidism are both associated with an increased fracture risk (Williams, 2009). Hyperthyroidism leads to uncoupling of the osteoclast-osteoblast mechanism, and a loss of up to 10% of bone mass per cycle (Bassett and Williams, 2003, Galliford et al., 2005). Although the exact mechanisms of TH on bone are as yet unknown (Williams, 2013), It has been demonstrated that there is an interaction of TH and the sympathetic nervous system, which controls bone mass in adult mice (Fonseca et al., 2014).

Findings have shown that the effects of adult-onset GH deficiency (AGHD) on bone can be treated by at least one year's supplementation with GH, which causes gains in BMD in males (Burman et al., 1997). The efficacy of GH treatment in females with AGHD has not been sufficiently explored, with the resultant warning that GH treatment in females should take into account the effects of any current hormone replacement therapy on IGF-1 production (Yamamoto and Sugimoto, 2014). In contrast to the effects on children, it has been shown that the combination of GH and testosterone in adult men did not have additive effects on bone structure beyond those of testosterone treatment alone (Mukaddam et al., 2014).

PTH stimulates osteoblast proliferation and activation; higher levels of PTH have been shown to be associated with lower BMD in adult men and women (Fujiyoshi et al., 2013). Vitamin D is necessary for the body to correctly absorb and use calcium; a decrease in serum vitamin D and an increase in PTH has been shown to be associated with decreases in BMD (Terzi et al., 2010). It has been shown that serum PTH is significantly correlated with markers of bone turnover (Xiao et al., 2014). Previous findings have shown that there is a significant effect of PTH on the rate of bone formation and mineralisation (Smith et al., 2014).

Oestrogen has been shown to regulate bone health in physically active adults, both men and women (Krassas and Papadopoulou, 2001, O'Donnell and deSouza, 2011, Smith et al., 1994). This occurs through stimulatory effects of the osteoblasts, accompanied by inhibitory effects on osteoclasts (see 2.1.7.1). Testosterone also plays a role in bone health in men, by reducing bone turnover (Katznelson et al., 1996). It has been shown that there is an increase in bone mass following testosterone treatment in adult men (Behre et al., 1997, Mukaddam et al., 2014).

It has been shown (Johnell and Kanis, 2005, Schnatz et al., 2010) that a number of factors related to the reproductive system influence BMD in women. These factors include parity, age at menarche and at menopause, duration of menopause, age at first pregnancy, and duration of breast-feeding. During pregnancy, the maternal body secretes large amounts of oestrogen and progesterone, both of which have positive effects on bone mineral density. However, there is no clear evidence that there is an increase in maternal bone mineral density following pregnancy; it appears that the opposite effect is true (Ersoy et al., 2015, Kovacs and Kronenberg, 1997, Naylor et al.,

2000). It is suggested that the decrease in maternal bone density during pregnancy is due to the increased calcium demand on the skeleton from the growing child (see 2.5.1).

Amenorrhoea is a condition in which normal menstrual function is disordered. It is defined as missing three to six consecutive menstrual cycles, and can occur as a result of low energy intake or high energy expenditure. Low energy intake in comparison to expenditure results in low levels of body fat, a decrease in the release of Gonadotropin-releasing Hormone, the hormone which starts the menstrual cycle, and a subsequent cessation of regular menstrual cycles (Gordon 2010). Amenorrhoea is associated with loss of trabecular bone and BMD, and so an increased risk of developing osteoporosis (Cook et al., 1987). The female athlete triad is a condition in which low energy intake/high energy expenditure, amenorrhoea, and low BMD are present (Nattiv et al., 2007). This syndrome is exhibited by non-athletes as well as by athletes, particularly by those participating in leanness or aesthetic sports (O'Connor et al., 1996, Sundgot-Borgen and Torstveit, 2004). Athletic amenorrhoea, the occurrence of amenorrhoea due to large amounts of exercise, is more common in weight-restricted sports such as running and gymnastics (Sanborn et al., 1982, Torstveit and Sundgot-Borgen, 2005b). Athletic amenorrhoea is associated with low BMD in both the axial and appendicular skeleton (Myburgh et al., 1993, Zanker et al., 2004), although athletic amenorrhoeic women show higher BMD than their non-athletic counterparts, suggesting a protective role of exercise in BMD (Bass et al., 1993, Rigotti et al., 1984, Seeman et al., 1992).

2.1.7.3 Hormonal basis of ageing bone loss

As individuals age, there is a change in the circulating hormones within the body such as PTH. PTH levels increase with age, leading to increased bone resorption. GH levels decrease with age (Finkelstein et al., 1972). Although, given adequate dietary intake and sunlight exposure, there is no change in vitamin D levels within the ageing body, it has been suggested that there is a higher demand for vitamin D in older adults in order to combat the effects of hyperparathyroidism (Vieth et al., 2003). As women undergo the menopause, there is a large decrease in the amount of oestrogen in the body. Although men do not undergo this rapid shift, there is a gradual decrease in the levels of androgenic hormones in the body as they get older, both oestrogen and testosterone

(Frank, 2003, Greendale et al., 1997). In aging men, chronological age is the most predictive factor of bone loss (Glynn et al., 1995, Kirchengast et al., 2001).

High levels of thyroid hormone, or hyperthyroidism, is associated with decreased BMD in men and women, and increased fracture risk in women (Cummings et al., 1995, El Hadidy et al., 2011). It has been demonstrated that the use of high levels of thyroid hormone ($>200\mu\text{g}$) results in significant decreases in BMD in older women, but that the use of oestrogen therapy in conjunction with thyroid hormone mitigates these effects (Schneider et al., 1994). It has also been suggested that serum thyroid stimulating hormone (TSH) may be associated with increased risk of hip fracture (Waring et al., 2013).

It has been shown that elderly women who have insufficient vitamin D during the winter months are at increased risk of hyperparathyroidism (Kim et al., 2012a); hyperparathyroidism, elevated levels of PTH in the blood, results in increased release of calcium from the bone matrix into the blood, and subsequently to a loss of bone mass. Supplementation with calcium and vitamin D in older men and women has been shown to result in significant reduction in bone loss as well as reducing nonvertebral fracture incidence (Dawson-Hughes et al., 1997). It has been demonstrated that supplementation with vitamin D alongside calcium is more effective at restoring parathyroid gland function than calcium alone (Pfeifer et al., 2001).

IGF-1 has been shown to play a role in the maintenance of adult bone mass, and it has been suggested that it may be used to treat osteoporosis in older adults (Xian et al., 2012). Results have shown that IGF-1 levels are significantly correlated with BMD in elderly women, although not in men (Langlois et al., 1998). It has also been demonstrated that circulating levels of IGF-1 are associated with bone growth in mice (Yakar et al., 2002).

Oestrogen has been shown to be the androgenic hormone most highly correlated with BMD in older adults, both women and men (Greendale et al., 1997). Findings show that oestrogen is the dominant androgenic hormone in determining bone resorption in older men, but that both oestrogen and testosterone are important in the maintenance of bone formation (Falahati-Nini et al., 2000). It has been shown that oestrogen resistance in men may cause osteoporosis (Smith et al., 1994), and that oestrogens are more

predictive of bone mass than free testosterone (Ongphiphadhanakul et al., 1998). Low sex hormone levels in older men is a significant predictor of low BMD (Barrett-Connor et al., 2012, Kenny et al., 2000, Murphy et al., 1993). It has also been demonstrated that there is a significant relationship between testosterone and bone parameters, and an inverse correlation between bone parameters and sex hormone binding globulin (SHBG) levels (Kyvernitakis et al., 2013). As women undergo the menopause, there is a sudden decrease in the amount of oestrogen in the body. Given the effects of oestrogen on the bone cells (see 2.1.7.1), this decrease leads to a reduction in osteoblast activity, and a subsequent decrease in bone mass. In postmenopausal women, it has been shown that high levels of immature osteoprogenitor cells is associated with a slight decrease in BMD (Pirro et al., 2012). Findings suggest that long-term oestrogen therapy after the menopause may help to maintain bone density into later life (Felson et al., 1993).

There is some conflicting evidence surrounding the effects of certain hormones on bone in older adults; it has been suggested (Dennison, 1999) that calcitropic and reproductive hormones were not predictive of bone loss in a cohort of older men and women. It is possible that there was no correlation between sex hormone levels and bone loss, but that there was a correlation between sex hormone level and peak BMD, meaning that the sex hormone level would still be a predictor of osteoporosis risk. In addition, the DXA used was changed between baseline and follow up scans; although there was cross-machine validation, this could explain the lack of correlation between sex hormones and bone loss (Dennison, 1999). Given the large volume of research supporting the relationship between androgenic hormone levels and BMD, it can be concluded that differences in methodology may be responsible for the lack of relationship found in this study.

2.2 Exercise and Bone Health

2.2.1 Mechanical loading of bone

Bone turnover is a process which occurs throughout life. Wolff's Law, a theory developed by Julius Wolff, and the subsequently developed Mechanostat theory, state that bone is able to adapt to loads placed upon it through altered bone turnover (Frost, 1994). High bone loading results in the formation of micro-fractures within the microarchitecture of the bone; as these fractures are healed by the osteoblasts, the bone

is strengthened along the lines of stress. The bone therefore becomes stronger in the direction of the applied loads. Regular participation in impact exercise results in bone loading, and therefore an increase in bone formation, and bone strength. In addition to this, regular participation results in increases in muscle mass. Because muscles pull on the bone at attachment sites during activities of daily living as well as during exercise, increased muscle mass results in increased pull of the muscle on the bone. The muscle-bone unit describes BMC as a function of muscle development (Schoenau, 2005); increased pull of muscle on bone results in increased bone loading, and so an increase in bone formation and in bone strength. Osteocytes act as mechanotransducers; following mechanical loading, osteocytes release osteoclastogenesis inhibitors, with a resultant decrease in bone resorption (You et al., 2008). Mechanical stress has also been shown to directly stimulate osteoblast activity, with subsequent increases in the rate of bone formation (Gerber et al., 2008, Gerber et al., 2005).

Physical inactivity, such as that experienced through prolonged bed-rest, has been shown to be associated with reduced bone density alongside muscle wastage (Rittweger et al., 2005, LeBlanc et al., 1992); bone loss is seen after only 5 weeks of bed rest (Berg et al., 2007), and is re-accumulated slowly (Bloomfield, 1997). Lack of weight-bearing exercise has been shown to be associated with reduced bone formation but unchanged bone resorption, and subsequent loss of bone mass, following space travel (Morey and Baylink, 1978, LeBlanc et al., 2000, Caillot-Augusseau et al., 1998, Smith et al., 2005, Caillot-Augusseau et al., 2000).

The loss in BMD which happens during long-term flights in space has been found to be attenuated by resistance exercise (Smith et al., 2012), and time spent undertaking physical activity was associated with increased BMD in adult men (Chastin et al., 2014). Undertaking physical activity after injury, and the amount of time spent in physical activity, has been shown to be related to bone health in male quadriplegics (Chain et al., 2012). Exercise in healthy active men has been shown to be beneficial to bone health (Eleftheriou et al., 2013). It has been demonstrated that physical activity in older men is associated with attenuation of bone degradation (Warden et al., 2014), and with greater bone quality and decreased fracture risk (Furrer et al., 2014). However, physical activity levels have not been shown to affect markers of bone metabolism in haemodialysis patients (Morishita et al., 2014).

It has also been shown that sedentary behaviour in adult women is associated with bone degradation, and that exercise has a “protective” effect on bone (Chastin et al., 2014). Active men and women have been shown to have significantly greater BMD than their sedentary counterparts (Sritara et al., 2015, Coaccioli et al., 2013). Physical activity changes have been shown to be associated with alterations in bone turnover makers and IGF-1 (Ardawi et al., 2012). Aerobic exercise in female breast cancer patients has been found to attenuate loss of bone mass and strength (Hojan et al., 2013). Physical activity in premenopausal women has been shown to be positively correlated with BMD (Kim et al., 2012b). However, it has been found that physical activity did not seem to affect BMD in women (Sritara et al., 2015). It has been concluded that physical activity throughout life is important for optimal bone health (Specker and Minett, 2013, Emaus et al., 2014).

Exercise in rodents has been shown to be promote measures of skeletal health (Novotny et al., 2012, Warden et al., 2013). Children have exhibited increased BMD following weight-bearing physical activity (Meiring et al., 2013), and weight-bearing exercise in girls has been shown to be associated with increases in BMD of the lumbar spine; the effects of exercise appear to be site-specific (Ishikawa et al., 2013). It has also been demonstrated that long-term exercise in prepubertal children results in increases in bone mass, regardless of gender, and in bone size in girls (Detter et al., 2014, Detter et al., 2013). In addition to this, exercise in young people has been shown to be beneficial for bone health throughout life (Warden et al., 2014, Warden and Mantila Roosa, 2014, Herrmann et al., 2012).

Different forms of exercise have been shown to have different physiological effects on bone mass; non weight-bearing activities such as swimming and cycling have been demonstrated to have no positive effect on bone mass in comparison to a sedentary lifestyle (Duncan, 2002; Nichols, 2003; Taaffe, 1995). There is also some evidence to suggest that endurance running may not have beneficial effects on bone mass (Hetland, 1993). Resistance exercise has been shown to have a positive effect on bone mass maintenance and accrual in a variety of populations, and so is recommended as contributing towards bone health (Blimkie et al., 1996, Kerr et al., 2001, Lohman et al., 1995a, Maddalozzo and Snow, 2000, Nichols et al., 2001).

2.2.2 Exercise and peak bone mass accrual

Wolff's Law states that, through remodelling, bones adapt to loading through increased strength in the direction of the loading (Ruff et al., 2006). As an individual undergoes weight-bearing physical activity, the bones are loaded due to ground-reaction forces and muscle forces, causing microfractures to the bone matrix. Damaged bone is removed by the osteoclasts, and replaced with new bone, formed by the osteoblasts (see 2.1.1). Osteocytes have been shown to be responsive to fluid flow (Weinbaum et al., 1994); it has been demonstrated that mechanical strain causes fluid flow within the canalicular network (Knothe Tate et al., 2000). It is therefore surmised that loading on the bones causes fluid changes within the bones which are detected by the osteocytes (Burger and Klein-Nulend, 1999). Osteocytes then release signalling molecules which stimulate osteoblast and osteoclast activity. This mechanism of bone remodelling means that the effects of bone loading are site-specific; the gains in bone mass are apparent only in the bones which were loaded. For example, it has been demonstrated that partaking in racquet sports results in an increase in bone mass in the playing arm over the non-playing arm (Kannus et al., 1995).

Physical activity in early life causes bones to become denser, causing peak BMD to be greater. Physical activity has been shown to affect both cortical and trabecular bone in long bones in prepubertal girls. Increased levels of physical activity lead to increased density of the cortical and trabecular bone, and an increase in the size of the cortical bone (Michalopoulou et al., 2013). In addition to this, it has been found that lean mass is predictive of bone mineralisation in prepubertal males and females, and that weight bearing physical activity is positively correlated with BMD in prepubertal and adolescent boys (Baptista et al., 2012, Boot et al., 1997, Lima et al., 2001). Physical activity during childhood and adolescence has been shown to result in significantly increased bone formation (Bailey et al., 1999), and improved bone health in later life (Cooper et al., 1995, Herrmann et al., 2012, Slemenda et al., 1991, Warden and Mantila Roosa, 2014, Warden et al., 2014). Physical activity is important for bone mass accrual in prepubertal male and females; intervention at this stage of life may influence bone mass and fracture risk in later life (Jones and Dwyer, 1998).

Physical activity in girls has been shown to be associated with gains in BMD and in bone strength in weight-bearing bone (Farr et al., 2013). However, elite athletes who

compete in non-weight-bearing sports such as swimming and cycling do not exhibit increased bone density in comparison to sedentary individuals despite high skeletal muscle mass (Cassell et al., 1996, Duncan et al., 2002, Fehling et al., 1995, Orwoll et al., 1989, Risser et al., 1990), suggesting that exercise has a direct effect on bone remodelling, independent of the effect of increased muscle mass on bone loading.

Weight-bearing physical activity has beneficial effects on bone, both directly and indirectly through changes in the muscle, as described in the Mechanostat theory and the muscle-bone unit (Gerber et al., 2005, Gerber et al., 2008, Schoenau, 2005). Combined resistance and high impact weight-bearing exercise training programs have been shown to promote beneficial changes in bone health in pre- and postmenopausal women (Kelley et al., 2013, Nikander et al., 2010a, Wolff et al., 1999).

Although it has been reported that beginning physical activity before the onset of puberty results in greater gains in bone mass accrual than beginning after puberty (Kannus et al., 1995), beginning habitual physical activity at any stage through childhood and adolescence yields beneficial results. Short-term exercise training (5-8 weeks) in adolescents results in increased biomarkers of bone formation (Eliakim et al., 1996, Eliakim et al., 1997, Woitge et al., 1998), showing increased osteoblast activity.

2.2.3 Exercise and attenuation of bone loss

In older females, an association has been shown between high physical activity levels and low fracture risk, and an association has been shown between higher strength and higher bone quality, and a subsequent decrease in fracture risk (Furrer et al., 2014). It has also been shown that physical activity in older adults provides protection against bone degradation (Warden et al., 2014). It has been suggested that increases in physical activity can help protect against low BMD and related diseases (Muir et al., 2013).

Physical activity in postmenopausal women attenuates loss of bone mass (Muir et al., 2013, Rikkonen et al., 2010, Svejme et al., 2014), results in increased bone mass (Calderon-Garcia et al., 2013) and improved skeletal health (Cheung and Giangregorio, 2012), while short-term physical activity interventions have been shown to improve bone quality (Tolomio et al., 2008). Some findings suggest that only moderate and high volumes of physical activity have a positive effect on postmenopausal bone health (Kim et al., 2012b, Wee et al., 2013, Zhong et al., 2012). It has been seen that

postmenopausal women show an especially high association between life-time physical activity and a decrease in bone stiffness, and a decreased risk of osteoporosis and osteoporosis-related fractures (Herrmann et al., 2012). Findings have shown that initiation of physical activity in postmenopausal women resulted in a decrease in bone resorption, and attenuation of bone loss (Bergstrom et al., 2012). Physical activity in postmenopausal women has been found to be associated with a decrease in hip fracture risk (Armstrong et al., 2011). However, it has been suggested that, in healthy postmenopausal women, there is only a minor effect of physical activity level on bone mass (Calderon-Garcia et al., 2013). There is also a suggestion that physical activity is not as strong a determinant of BMD as body composition is (Gaba et al., 2012).

Undergoing weight-bearing exercise results in prevention of bone loss in postmenopausal women (Snow et al., 2000, Smith et al., 1989a), and can result in stimulation of bone formation (Roghani et al., 2013) and even the reversal of bone loss (Krolner et al., 1983). Non-weight bearing exercise is not effective at attenuating this bone loss (Sinaki, 1989). With reduction of the weight-bearing exercise, bone formation does not appear to be maintained at the higher level, and bone mass returns to baseline levels (Dalsky, 1988, Hamilton et al., 2010). (See 2.2).

Association has been demonstrated between BMD and muscular strength of the forearm in postmenopausal women (Shin et al., 2014), supported by previous findings of association between BMD and muscle strength (Helge and Kanstrup, 2002, Henderson et al., 1995, Pasco et al., 2015). Associations have also been found between physical activity and a reduced risk of hip fracture in postmenopausal women (Armstrong et al., 2011, Armstrong et al., 2012). Despite these findings, physical activity was not shown to be associated with BMD changes in rural postmenopausal Japanese or Czech women (Gaba et al., 2012, Kitamura et al., 2011).

There is evidence that sedentary behaviour in older adult women is associated with degradation of the bone in the hip region (Chastin et al., 2014). Decreases in physical capabilities is correlated with an increase in risk of hip fracture (Lai et al., 2013). Sedentary behaviour is associated with poor bone health (Wee et al., 2013); it has been demonstrated that leisure-time physical activity is positively correlated with BMD in postmenopausal women (Kim et al., 2012b).

In conclusion then, exercise has been shown to exert a beneficial effect on bone health in osteoporotic women (Hamilton et al., 2010). Although exercise in earlier life results in increased bone density and strength in later life, physical activity is important for maintaining aging skeletal health (Cheung and Giangregorio, 2012). Regular exercise is recommended for postmenopausal women to help decrease the risk of osteoporosis (Svejme et al., 2014, Wee et al., 2013).

2.2.4 Exercise and bone health in premenopausal women

Undergoing regular exercise results in changes in the bone remodelling process. Trained individuals exhibit higher levels of bone formation markers (Jurimae et al., 2006, Hetland et al., 1993, Karlsson et al., 1995, Nishiyama et al., 1988, Bell et al., 1988) and higher BMD (Brahm et al., 1997) when compared to sedentary individuals. These results are not necessarily consistent in all individuals; amenorrhoeic females have lower levels of bone formation markers (Zanker and Swaine, 1998), due to the altered energy pathways. Highly trained female athletes have been shown to exhibit normal levels of bone formation markers (Ryan and Elahi, 1998), although it is possible that due to the high level of training these women were at risk of amenorrhoea, meaning that their bone turnover would be affected.

Undergoing physical activity after injury is associated with increases in bone density and bone markers (Chain et al., 2012, Sritara et al., 2015). Loss of bone mass is evident in individuals not performing any weight bearing activity; 6 weeks of non-weight-bearing has been shown to result in a loss of more than 1% of BMD in the affected limb. This loss was evident even after return to normal weight-bearing activity, with a loss of 2.5% of baseline BMD 13 weeks after return to normal activity (Kazakia et al., 2014). Bone turnover is a lengthy process, and measurable changes in BMD can take up to 9 months to appear. Biomarkers of bone formation and resorption are more responsive to change, but were not measured in the study cited.

Physical activity levels are an important factor in bone mass and BMD (Aloia et al., 1988). The sites which are most commonly affected by fragility fracture are the radius, the lumbar spine, and the neck of the femur. BMD at all of these sites is significantly correlated with physical fitness (Pocock, 1986). It has also been shown that increasing physical activity levels is associated with increases in hip and spine BMD (Kim et al.,

2012b, Aloia et al., 1988, Alekel et al., 1995) and biomarkers of bone formation (Lohman et al., 1995b). Conversely, there is evidence that sedentary behaviour in adult women is associated with degradation of bone in the hip (Chastin et al., 2014).

Aerobic, weight-bearing exercise has been shown to attenuate bone loss in adult women, and also to reduce body fat. Excess adiposity in adults has been shown to be associated with deterioration of muscle and bone (Ilich et al., 2014), and with poorer bone density (Ng et al., 2013), although these relationships appear to be dependent on factors such as age, gender, and menopausal status (Ng et al., 2013). As in children, lean mass has been shown to be a significant predictor of bone mass and bone geometry in adult women (Mallinson et al., 2013).

Resistance training was also found to attenuate loss of bone strength (Hojan et al., 2013). Meta-analyses have demonstrated that combined resistance and high impact exercise is optimal for eliciting bone health gains (Kelley et al., 2013, Nikander et al., 2010a, Wallace and Cumming, 2000, Wolff et al., 1999). Half of the strength gains which are achieved through 1 year of training are achieved during the first 12 weeks of a training program in older adults (Latham et al., 2004), suggesting that a short-duration program using resistance and high impact exercise may result in significant changes in bone and muscle.

2.3 Muscle Biology and Sarcopenia

Maximal strength peaks around the second to third decade of life, with a significant decrease in maximal strength occurring by the fifth decade (Hurley, 1995, Janssen et al., 2000). This strength decline is generally attributed to either decreased physical activity, or disease resulting in disuse. The severity of strength decline increases after the middle of the seventh decade (Abe et al., 2016). Sarcopenia, loss of muscle mass and strength, is not considered to be a disease state, although it is associated with loss of physical functioning, disability, and increased morbidity and mortality (Santilli et al., 2014).

Although muscle strength and muscle mass decline in conjunction with one another, the losses are independent of each other (Goodpaster et al., 2006). Muscle strength and mass do not decrease concurrently (Goodpaster et al., 2006), suggesting that strength

may be a greater indicator of dysfunction in the muscle; muscle strength is important for the maintenance of movement and mobility, and is a predictor of the functional decline which often accompanies ageing (Humphreys et al., 2002). Muscle weakness is associated with reduced walking speed, and an increased incidence of disability and falling, but can be improved through exercise (Schlicht et al., 2001).

Normal ageing results in changes in body composition; these changes include a decrease in muscle mass, and an increase in fat mass, due in part to altered substrate utilisation and a decreased resting metabolic rate; men have approximately a 5% decrease in resting metabolic rate per decade, and women a 3% decrease (Atlantis et al., 2008, St-Onge, 2005, St-Onge and Gallagher, 2010). Sarcopenia, loss of muscle mass and strength, results in frailty, disability, and a loss of independence in elderly adults. There is an increase in levels of obesity in adults over the age of 60 years, causing problems for healthcare practitioners seeking to decrease the risks associated with obesity, to preserve muscle mass, and to decrease the risk of disability (Ogden et al., 2012). Sarcopenic obesity results in decreased fat-free mass, decreased muscle quality, and a loss of physical functioning (Rolland et al., 2009, Stenholm et al., 2008).

Ageing affects many variables, all of which interact, influencing quality of life. These factors include lifestyle, genetics, and chronic disease development. Ageing is associated with decreased muscle mass and strength, reduced muscle power, reduced connective tissue elasticity, and loss of balance and flexibility. Together, these affect functional performance, which is required for independent living. Falls are a major source of morbidity and mortality in the elderly (Fuller, 2000, Rizzo et al., 1998), and more than 90% of hip fractures occur as a result of a fall (Dargent-Molina et al., 1996, Stevens and Olson, 2000). In the UK, approximately £3,496 million was spent on fracture treatment and care in 2010 (Svedbom et al., 2013).

However, these changes are alterable through intervention. Exercise, sport, and physical activity all have multiple effects on the human body. Exercise not only decreases the risk of certain diseases such as cardiovascular disease, heart disease, type 2 diabetes, and stroke, it helps to maintain healthy weight, and also has long-term effects on the muscle and bone.

2.4 Exercise and Muscle Health

2.4.1 Exercise and Muscle

Skeletal muscle is malleable, allowing the muscle to adapt to the demands placed upon it through remodelling. Adaptation of muscles to exercise occurs as a result of certain stimuli; these include stretch, contraction, and damage to the muscle fibres (Crewther et al., 2006, McDonagh and Davies, 1984). The plasticity of the muscle results in long-term changes in the muscle force, endurance capability and contractile velocity, as a result of the demands placed on the muscle (Flück, 2006). These changes allow adaptation of skeletal muscle to exercise training, improving performance through alterations in the specific performance of the contractile tissue. This response is specific to a given stimulus, a phenomenon known as “sports specificity”, where the adaptations of a particular athlete allows them to excel at their sport. Despite the specificity of adaptations, there is some carryover from training effects (Morrissey et al., 1995). Physiological adaptations are specific to the muscle actions involved, the muscles trained, the range of motion of the movement, and the energy systems utilised; in sports which use some of the same pathways, there is a carryover in the adaptation of some energy pathways (Hoff and Almåsbaek, 1995).

Structural adjustments within the muscle occur as a result of the accumulation of multiple transient adjustments following the repetition of exercise stimuli. These alterations are caused by changes in metabolic, mechanical, hormonal, and neuronal factors. The adaptation of the muscle to the stimulus is dependent on whether the dominant stimulus is the degree of loading of the muscle, or the number of muscle contractions which are performed (Bottaro et al., 2006, Goto et al., 2004). A high number of repetitions at a comparatively low load results in adaptations towards fatigue resistance, allowing the muscle to sustain a high number of slow contractions. Conversely, high loading with a comparatively small number of repetitions results in muscle hypertrophy, leading to an increased maximal force and muscle mass. The impact of contractile stimuli also allows maintenance of skeletal muscle mass and oxidative capacity, whereas inactivity results in deconditioning of the muscle function (Berg et al., 2007).

The onset of exercise causes changes in the working muscle. Sustained training results in physiological changes. It results in increased capillary density, increased size and

number of mitochondria, an improvement in the ability of the muscle to utilise fats and carbohydrates, and increased activity in the Krebs cycle, which is important for aerobic respiration (Perry et al., 2008, Bruce et al., 2006). One of the changes which happens in the working muscle is a change in Adenosine Triphosphate (ATP) homeostasis (see 2.6) (Wibom et al., 1992, Ingjer, 1979). After training, the onset of exercise results in smaller decreases in ATP and PCr, and subsequently smaller increases in ADP and Pi levels. Therefore, there is a slower rate of anaerobic metabolism systems such as glycolysis.

Endurance training results in an increase in the cross-sectional area of type 1, or “slow-twitch” muscle fibres, and an improvement in the ability of the body to utilise fat as a fuel source (Kiens et al., 1993, Carter et al., 2001). It also results in increases in the maximum cardiac output, and $\text{VO}_{2\text{max}}$, along with improved oxygen transport and delivery to the working muscle. This happens alongside changes in the substrate utilisation in the muscle, which results in increased endurance capacity.

Strength training and power training do not result in the same physiological changes as endurance training. They result in only small changes in aerobic capacity, but do result in changes in the ATP and PCr energy systems. It also leads to muscle hypertrophy, an increase in muscle mass, and an increase in maximum power output (Fielding et al., 2002, Macaluso and de Vito, 2004).

2.4.2 Body mass and exercise

Weight loss has been shown to have potential negative effects on fat-free mass, especially muscle and bone. Diet-induced weight loss has been shown to result in decreases in fat mass and fat-free mass; approximately 75% of weight lost through diet is thought to be from fat mass, with the remaining 25% lost from fat-free mass (Weinheimer et al., 2010). Diet-induced weight loss in older adults therefore worsens age-related losses of fat-free mass, resulting in greater physical function impairment. The American Society for Nutrition and Obesity Society suggest that quick weight loss can be achieved with a calorie reduction of $500\text{-}1000\text{kcal}\cdot\text{day}^{-1}$, and moderate loss through reduction of $500\text{-}750\text{kcal}\cdot\text{day}^{-1}$ (Villareal et al., 2005). The amount of weight lost during diet-induced weight loss does not appear to affect the proportion of fat-free

mass lost; energy restrictions below and within the recommended ranges result in similar changes in overall fat-free mass (Weinheimer et al., 2010).

Weight-bearing aerobic exercise accompanying diet-induced weight loss has been shown to decrease losses of fat-free mass from 25% of total weight lost to 12% (Garrow and Summerbell, 1995), therefore preserving muscle mass. This demonstrates that the addition of exercise to energy restriction is effective at preserving fat-free mass during weight loss in older adults. Exercise alone as a weight-loss aid results in loss of less body weight than diet-only, or diet plus exercise as weight loss aids, and also results in greater maintenance of fat-free mass than either diet alone, or diet in addition to exercise (Jakicic and Otto, 2006, Villareal et al., 2005).

Physical frailty is common in sarcopenic individuals, with increased prevalence in older adults (Rockwood et al., 1999). Older adults who suffer from increased fat mass and decreased muscle mass are classified as suffering from sarcopenic obesity; these individuals demonstrate increased functional impairment (Janssen et al., 2002). Although obese individuals have been shown to have greater absolute values of lower body fat-free mass than their healthy weight counterparts, there is a lower percentage of fat-free mass and a decreased muscle quality in these individuals (Roubenoff, 2004). Obesity is also associated with a number of physiological impairments, including decreases in physical performance, peak aerobic power, functional performance, strength, walking speed, balance, and health measures (Rockwood et al., 1999). In older adults, low Body Mass Index (BMI) is associated with disability; slightly overweight individuals may have a protective effect against disability. Obese older adults exhibit physical frailty, with decreased physical function (Janssen et al., 2002). Moderate energy restriction accompanied by exercise appears to be effective for counteracting sarcopenic obesity in older overweight and obese adults, while protecting against loss of fat-free mass (Frimel et al., 2008).

Men and women exhibit different hormonal changes with ageing, and different physiological changes to energy restriction and exercise. Decreased sex steroids, oestrogen, growth hormone, and testosterone, all influence fat-free mass. Due to the greater changes in hormones in older women, older men exhibit greater absolute and relative increases in muscle mass (Ivey et al., 2000) following exercise training. However, a recent meta-analysis (Peterson et al., 2010) showed that there was no

association between gender, age, and strength effects, implying that there is an adaptive response to exercise in both men and women, across all decades of adult life.

Significant strength adaptations to exercise can be seen in very old adults, although early strength accrual results in greater preservation of functional performance (Sayer et al., 2008). This results in greater maintenance of capacity for activities of daily living and independence, and prevention of disability (Peterson et al., 2010). Significant variation is seen in muscle weakness and atrophy in older individuals, which can be associated with the amount of muscle mass and strength accrued in earlier life (Sayer et al., 2008).

2.4.3 Physical inactivity

Physical inactivity is associated with increased susceptibility to chronic diseases, and decreased quality of life, in older individuals (Chodzko-Zajko, 2014). Given the potential of regular exercise, in particular resistance training, to improve multiple measures of quality of life, guidelines for improving health and fitness measures include the addition of resistance training in exercise programs (Garber et al., 2011). Use of a variety of exercises is recommended, and at an intensity sufficient to stimulate the development and maintenance of muscular strength and endurance, and lean body mass. Exercising 2-3 times per week is sufficient to promote training adaptations in healthy individuals (Hass et al., 2001).

Despite the association of physical inactivity with adverse health effects, most individuals do not attain sufficient levels of physical activity. A recent report by the European Union found that 42% of adults did not take part in any regular exercise, and 30% did not take part in any regular forms of physical activity (EuropeanCommission, 2014). There is a decrease in physical activity levels with age, with 58% of individuals aged 55 or over not taking part in any regular exercise (EuropeanCommission, 2014). Elderly adults demonstrate declines in lean body mass and functional capacity, and increases in inactivity and disability with age (Villareal et al., 2004).

2.4.4 Training Program Design

Regular exercise including resistance training can attenuate age-related declines in body composition, and reduces the incidence of falls and fall-related injuries (Carter et

al., 2012). Resistance training also attenuates the onset of sarcopenia, improves endurance and metabolic rate, reduces insulin resistance, body fatness, loss of bone density, pain, and loss of function, and can reduce blood pressure as well as increasing musculoskeletal fitness and athletic performance, and preventing and improving rehabilitation of orthopaedic injuries (Feigenbaum and Pollock, 1999). Regular participation in resistance training decreases the rate-pressure product when lifting, therefore decreasing cardiac demand during daily activities such as carrying shopping (Pollock et al., 2000). Increases in strength result in improved functional performance, maintenance of independence, and prevention of disability, therefore improved measures of daily activities (Aniansson et al., 1980). Resistance training also improves health factors associated with the development and onset of chronic disease, and has been shown to improve muscle mass and strength in older as well as in younger individuals (Peterson et al., 2011). Resistance training results in increases in lean mass and decreases in fat mass as well as increases in functional muscle strength (Seynnes et al., 2004, Skelton et al., 1995).

ACSM recommends 150-300min.week⁻¹ moderate intensity aerobic exercise, plus at least 2 days.week⁻¹ moderate-to-vigorous resistance training, plus at least 2 days.week⁻¹ flexibility training for all older adults, regardless of obesity status (Garber et al., 2011). The inclusion of stretching, aerobic, and strength exercises results in improved flexibility, endurance, and strength measures. This enables maintenance of functional capacity, and prevention and management of chronic diseases.

Resistance exercise may be effective at improving functional performance; short bouts of resistance training in older adults has been shown to result in increased protein synthesis and neuromuscular adaptations (Phillips et al., 1997). Despite lower pre-exercise rates of protein synthesis in comparison to younger cohorts, older adults exhibit similar post-exercise levels (Holviala et al., 2006). These findings suggest that disuse or inactivity may contribute to ageing muscle weakness, alongside mitochondrial dysfunction and associated problems such as decreased utilisation of amino acids (Johnson et al., 2013).

Increasing resistance intensity has a significant positive association with strength measures of the upper and lower body; higher intensity resistance exercise promotes greater improvements in strength. Higher intensity training is associated with increased

strength gains in older populations in comparison with low and moderate intensity exercise (Seynnes et al., 2004). Progressive resistance training has been shown to result in significant increases in strength, the most predictive measure of functional impairment (Skelton et al., 1995). The stimulus of resistance exercise results in muscle growth and maintenance in younger and older adults. Older individuals show significant association between resistance exercise and strength of the upper and lower body (Skelton et al., 1995). Resistance exercise can be effective at treating and preventing age-related declines in muscle function, and may reduce the likelihood of disability.

Physical frailty in older adults can be improved following exercise intervention. Progressive resistance exercise including flexibility, endurance, and strength training minimises loss of fat-free mass while improving physical functioning. This training therefore improves measures of frailty in old and frail adults. Weight loss alongside resistance exercise improves measures of frailty in obese older adults (Villareal et al., 2006). Exercise without energy restriction is less effective as a weight loss strategy; although exercise alongside energy restriction may not result in greater weight loss than energy restriction alone, it protects against loss of fat-free mass (Ballor and Poehlman, 1994) (see 2.4.2). Resistance exercise stimulates muscle hypertrophy, and protects against fat-free mass loss more than aerobic exercise alone (Walberg, 2012).

Resistance training is the most effective method for both maintaining and increasing lean body mass in younger and in older adults (Peterson et al., 2011). Resistance training also improves muscular strength and endurance. The “progressive overload principle” encourages increasing the intensity of training and increasing the adaptations to exercise through increasing either the frequency or duration of activity, or both. The volume of training allows multiple adaptations within the body; neural, hypertrophic, metabolic, and hormonal responses (Ahtiainen et al., 2003, Kraemer and Ratamess, 2005). Alteration of training intensity can be achieved through varying the weight load, the number of sets and/or repetitions, and the rest interval between sets and between training bouts. The specificity of training adaptations means that the adaptation of the body is specific to the training stimulus, although there is some carryover in the training effects (Hoff and Almåsbaek, 1995, Morrissey et al., 1995).

A combination of single- and multiple-joint exercises used within resistance training allows isolation of specific muscles (through single-joint exercises), and increases in overall muscle strength (through multiple-joint exercises). Training bouts which last for 45-60 minutes, 2-3 times per week, increase participant adherence (Hass et al., 2000), and so are associated with increased adaptation to the exercise stimulus, and improvements in multiple organ systems. Recommendations are to starting exercise at low intensity and increasing intensity slowly, using slow, controlled movements through a pain-free range of movement, and maintaining a normal breathing pattern (Pollock et al., 1998, Garber et al., 2011). Progressive resistance training, with progression to heavier weights being introduced every 1 to 2 weeks, has a significant positive effect on muscle strength (Pollock et al., 1998, Garber et al., 2011). Resistance training results in strength gains even in older individuals, where exercise intensity has been shown to have a greater effect on strength gains than exercise duration (Latham et al., 2004).

Wolff's Law and the Mechanostat theory state that impact on the bone causes the bone to adapt and strengthen. Weight-bearing and high impact physical activity therefore cause bones to strengthen along the lines of stress, both due to direct impact on the bone and due to indirect effects through the muscle-bone unit (Gerber et al., 2005, Gerber et al., 2008, Schoenau, 2005). Recent meta-analyses have shown that combined resistance and high impact weight-bearing exercise training programs have been shown to promote beneficial changes in bone health in pre- and postmenopausal women (Kelley et al., 2013, Nikander et al., 2010a, Wolff et al., 1999).

The frequency of training within training programs requires that the rest period between training bouts be sufficient to allow muscle recovery and development (Bickel et al., 2005); however, extended periods of rest in between training bouts can result in detraining effects. A 48 hour rest in between training bouts is generally recommended (Bickel et al., 2005, Garber et al., 2011, Pollock et al., 1998), meaning that training 3 days per week is common. Training 2 days per week results in up to 90% of the training adaptations achieved through training 3 days per week in untrained individuals (Demichele et al., 1997), while benefits from resistance training can be maintained through resistance training only one day per week (Graves et al., 1990). The effects of training for only 2 days per week in untrained individuals gives muscles more time for

recovery in between training bouts, and is less time consuming, therefore increasing adherence to the exercise program.

2.5 Diet for Muscle and Bone Health

2.5.1 Calcium

The high amount of calcium in the bones means that it is important that children consume enough calcium in their diet to maintain the levels in the bone (see 2.1.2). However, other nutrients also need to be consumed in order to allow the body to absorb and use calcium. Cow's milk allergy sufferers, typically ingesting less calcium, have been shown to have an increase in OPG, suggesting disturbances in the balance of bone turnover (Ambroszkiewicz et al., 2013), and calcium intake has been shown to be positively correlated with BMD (Aton et al., 2014). It has been shown that consumption of dairy products earlier in life may improve BMD in adult women (Wadolowska et al., 2013).

Insufficient calcium in the diet leads to leeching of calcium from the bones in order to maintain levels in the blood. This can result in insufficient amounts of calcium hydroxyapatite in the bones. During pregnancy, women have a higher demand for calcium; the calcium needed for the growing baby is taken from the mother's diet and skeleton. This is met by an increase in vitamin D release, and subsequent increased calcium absorption in the small intestine. However, results have suggested that there is a negative bone balance during pregnancy, and that the increase in calcium absorption is not sufficient to meet the increased demand. During the third trimester of pregnancy, there is an increase in bone formation which is accompanied by an increase in IGF-1 (Moller et al., 2013). The maternal skeleton has been shown to recover bone density in the postpartum period (Møller et al., 2012, Sowers et al., 1995).

During pregnancy and lactation, calcium is supplied to the baby and the breast milk at the expense of the mother; maternal calcium appears to be transferred from the mother to the child, with the result that the mother loses bone mass (Tsvetov et al., 2014). However, this bone loss is only temporary, and the bone is rebuilt during the postpartum period. It has been demonstrated (Ersoy et al., 2015) that, with shorter interpregnancy intervals, there is a higher resultant risk of developing postmenopausal osteoporosis. It is suggested that this increased risk may be due to the maternal skeleton

being unable to fully recover lost bone mass if the interval between subsequent pregnancies is too short.

2.5.2 Vitamins

2.5.2.1 *Vitamin D*

Vitamin D enables the body to correctly absorb calcium from the intestines. Vitamin D can be taken into the body in one of two ways; it can be synthesised naturally by the body, or it can be taken in through the diet from foods such as oily fish and eggs or from dietary supplements. The rate of synthesis is increased when the skin is exposed to sunlight. Vitamin D deficiency can lead to insufficient levels of calcium in the bones; in children, it can lead to rickets. This is caused by a decrease in the mineralisation of the organic bone matrix, and is characterised by bowed bones and inflamed joints. Vitamin D intake has been shown to be positively correlated with BMD (Aton et al., 2014, Ubesie et al., 2013). Vitamin D deficiency during pregnancy has been shown to result in reduced accrual of bone mass in the child (Javaid et al., 2006). A lack of vitamin D during the winter months in children leads to an increase in bone resorption during those months, even despite sufficient calcium intake (Rajakumar et al., 2014). It has also been shown that in prepubertal children there is an association between vitamin D levels and BMD, with taller children with higher BMC having higher vitamin D levels (Vatanparast et al., 2013). Previous findings have revealed that there are gender differences in the skeletal growth phase, even pre-puberty, but vitamin D binding protein levels is associated with bone mass increases during puberty in both genders (Pekkinen et al., 2014).

Because of the need for vitamin D for calcium absorption and use, insufficient vitamin D consumption in adults can lead to "adult rickets", or osteomalacia, which is a softening of the bone from calcium depletion. Insufficient vitamin D consumption during the winter leaves elderly women at risk of bone loss (Kim et al., 2012a). Vitamin D deficiency can also be seen in adults with digestive disorders, who are unable to absorb the fats which vitamin D is soluble in. Serum vitamin D has been shown to be inversely associated with BMD in healthy adult men and women (Fujiyoshi et al., 2013). Premenopausal women have been shown to exhibit significant correlation between vitamin D levels and BMD (Yeap et al., 2012).

It has been found that deficiency of calcium, protein, and vitamin D is common in the elderly (Bischoff-Ferrari et al., 2004). This is due to the common decrease in overall caloric intake which is often associated with a decrease in physical activity levels. Although this decrease in caloric intake may not cause a direct effect on the bone, the associated decrease in intake of calcium, vitamin D, and protein, has a large impact.

2.5.2.1.1 Dietary supplementation

Recent findings have shown that supplementation of postmenopausal women with a mixture of calcium, vitamin D, inulin and soy isoflavones resulted in an increase in the absorption of calcium, and a subsequent increase in bone growth (Bevilacqua et al., 2013). It has been shown that low calcium intake in older adults is significantly associated with low BMD, however, the effects of low calcium intake could be normalised following an increase in vitamin D intake (Kim et al., 2014). It has been found that the combination of vitamin D and calcium supplementation in older adults results in a decrease in the risk of non-vertebral fracture (Hill et al., 2013), and in reduced bone loss and fracture incidence (Dawson-Hughes et al., 1997). Although serious adverse effects have been previously reported following calcium supplementation (Bolland et al., 2010, Bolland et al., 2008), recent analyses have shown that calcium supplementation is safe, with no severe adverse effects (Avenell et al., 2014). The recommended dietary allowance (RDA) of calcium is $1,000\text{mg}\cdot\text{day}^{-1}$ in adults; ensuring consumption of sufficient calcium is advice often given to individuals with low bone density (IOM, 2011). Despite the effects of vitamin D supplementation on BMD, it has been demonstrated that high vitamin D intake is not associated with an increase in bone mineralisation in osteoporotic bone, but is associated with a decrease in bone resorption (Lani et al., 2014). It has been shown that low vitamin D levels in older men causes poor health following low androgenic hormone levels to be more pronounced (Barrett-Connor et al., 2012). The RDA of vitamin D is $15\text{ }\mu\text{g}\cdot\text{day}^{-1}$, which is recommended for optimum bone and muscle health (IOM, 2011).

2.5.2.2 Other Dietary Vitamins

Bone health in adult women has been shown to be associated with a combination of dietary factors (Wadolowska et al., 2013). It has been suggested that adult women

should be encouraged to eat large amounts of fruits and vegetables, which appear to inhibit bone resorption (Gunn et al., 2013).

Vitamin C is necessary for the bones because it is used in the synthesis of collagen by the osteoblasts. Vitamin C is a cofactor for enzymes which perform the hydroxylation of two amino acids, proline and lysine, within the collagen precursor, procollagen. Deficiency of vitamin C leads to impaired collagen synthesis; the old collagen is broken down, but is not replaced by new collagen. Deficiency of vitamin C in children can lead to bone and tissue disorders such as tooth loss, impaired wound healing, and growth retardation (Carr and Frei, 1999). Vitamin C deficiency during pregnancy may result in low maternal weight gain, impaired function of the placenta, and impaired foetal development (Schjoldager et al., 2015).

2.5.3 Dietary Restrictions

2.5.3.1 Energy restriction

A calorie-restricted food diet has been shown to lead to weaker bones, along with inhibited growth and trabecular bone density (Pando et al., 2014). It has been found that prepubertal obesity is associated with an increase in bone mass, resulting from changes in bone formation rather than bone resorption (Gajewska et al., 2013). This increase in bone density accompanying increases in fat mass could be due to a number of mechanisms; firstly, the increased mass results in increased force acting on the bones. According to Wolff's Law (see 2.2), this increased stress on the bones would result in increased bone formation, and a subsequent increase in bone mass. In addition to this, body fat influences oestrogen levels through 4 different mechanisms (Frisch, 1987). Firstly, adipose tissue converts androgens to oestrogen (Nimrod and Ryan, 1975, Siiteri, 1981). Secondly, body weight influences the 'potency' of the oestrogen produced, with leaner women producing less 'potent' oestrogen (Schneider, 1983). Thirdly, obese females have decreased binding of oestrogen to SHBG, resulting in increased serum oestrogen (Apter, 1984, Siiteri, 1981), and fourthly, adipose tissue can store hormones (Kaku et al., 1969).

An energy restricted diet has been shown to result in decreased bone formation, and subsequent decrease in bone strength and BMD in obese rats (Shen et al., 2013). A negative relationship between net energy intake and bone formation has also been

demonstrated in physically active humans (Zanker and Swaine, 2000, Zanker and Cooke, 2004). Severely energy restricted diets in active females may lead to athletic amenorrhoea (see 2.1.7.2).

It has been demonstrated that an increase in BMI in older adults was associated with a decrease in testosterone in males, and a decrease in serum vitamin D in females, pre- and postmenopausal. Despite these changes, higher BMI was associated with higher BMD (Puntus et al., 2011), implying that there may be a protective effect of adiposity on BMD in older adults, in contrast with some previously mentioned findings in children. In addition to this, it has been demonstrated that higher BMI in postmenopausal women appears to protect against low BMD (Wee et al., 2013, Armstrong et al., 2011). This protective effect of adipose tissue on bones may be due to increased bone loading from the increased mass, or it may be due to the effects of adipose tissue on the body's stores of oestrogen (see 2.1.7).

In contrast to these findings, it has been suggested that a high fat diet is associated with a decrease in bone mass, particularly in immature bone (Inzana et al., 2013, Radetti et al., 2014). It is possible that these latter studies were confounded by the effects of physical activity; low levels of physical activity would result in increased adiposity, and, according to Wolff's Law, in decreased BMD (see 2.2). The effects of low levels of physical activity may have counteracted the effects of the adipose tissue and oestrogen levels.

2.5.3.2 *Gluten-free Diet*

Individuals suffering from coeliac disease can experience a deficit in bone; more serious sufferers have a greater deficit, evident in low trabecular and cortical density (Zanchetta et al., 2015). It has been demonstrated that a gluten-free diet is associated with higher BMD levels, and BMD positively correlated with vitamin D intake in coeliac patients (Margoni et al., 2012). These findings were opposed by earlier work, which found a detrimental effect of a gluten-free diet on bone mineralisation, but that early diagnosis of coeliac disease and keeping to a strict diet resulted in normal bone mineralisation (Mora et al., Scotta et al., 1997). Because undiagnosed coeliac disease results in damage to the villi on the small intestines, it can result in inhibited absorption of nutrients such as calcium and phosphorus. This may explain why early diagnosis and

strict diets helps maintain bone mineralisation, as it prevents intestinal damage and so nutrients such as calcium are properly absorbed. There is also evidence that children and adolescents who suffer from coeliac disease are at risk of vitamin D and K deficiency, which could result in poor bone health (Mager et al., 2012); this again could be attributable to villi damage, emphasising the importance for early diagnosis and strict diets.

2.5.4 Protein

Protein is necessary in the diet for the growth of new tissues, and the repair of old tissues. It accounts for approximately 50% of bone volume, and 30% of bone mass (Heaney and Layman, 2008), making it important to obtain sufficient dietary protein to maintain bone mass as the bone undergoes remodelling. Findings suggest that dietary protein has a beneficial effect on bone density, although it is unclear whether protein source (e.g. animal or vegetable) affects the efficacy of the protein (Jesudason and Clifton, 2011). A high protein diet has been found to be beneficial for bone health over a normal protein diet, even though it results in an increase in some dietary acids (Nebot et al., 2014).

Protein intake also plays a key role in determining circulating levels of IGF-1 (Fontana et al., 2008); it has been found that a low protein diet can result in a delay in muscular and skeletal growth by causing a decrease in IGF-1 (Fournier et al., 2014). The effect of physical activity on bone density in young and adolescent boys has been shown to be augmented by the consumption of a high protein diet (Chevalley et al., 2014), and that the beneficial effect of protein on bone is independent of gender (Libuda et al., 2011). It has been suggested that, in children without nutritional deficiencies, protein intake was the largest dietary factor in bone status (Libuda et al., 2011). Despite the large body of evidence supporting the role of protein in bone turnover, there is some disagreement in the literature; it has been found that protein consumption did not result in changes in bone resorption in children (Dalskov et al., 2014), and suggested that it is necessary to consider dietary acid as well as dietary protein when considering effects on bone (Remer et al., 2014), although it has been argued that the acidity of protein sources is negligible in comparison with the acidity of fruits and vegetables (Heaney and Layman, 2008).

High protein diets have been shown to cause a reduction in bone turnover with a subsequent increase in BMD; it is suggested that this may be attributable to altered intestinal calcium absorption (Gaffney-Stomberg et al., 2014). There is some evidence that, for protein to have a beneficial effect of protein intake on bone, it needs to be accompanied by sufficient calcium and vitamin D consumption (Mangano et al., 2014). This could be due to calcium constituting such a large part of the bone matrix, and so being necessary for bone remodelling. It has been suggested that, in adult women, a high protein diet is associated with greater bone strength, whereas for adult men a more refined diet may be detrimental for bone health (Whittle et al., 2012). Over time, the body may be able to respond to a low protein diet by altering hormone status, attenuating the impacts of low protein intake on IGF-1 and skeletal growth (Fournier et al., 2014).

Protein also plays a key role in maintaining musculoskeletal function, and it also decreases the prevalence of bedsores, anaemia, and lung and renal infections as individuals age (Rizzoli and Bonjour, 2004). However, there is some suggestion that high levels of protein intake can cause “leeching” of calcium from the bones, resulting in calcium depletion (Ginty, 2003). It has been suggested that a high dietary protein intake doesn’t directly affect bone density, but that it slows bone turnover (Jesudason et al., 2013). As individuals age, there is an attenuation of the anabolic response to protein; it is therefore suggested that older adults should increase protein consumption in order to prevent fragility fractures (Bonjour, 2011). However, a recent meta-analysis has concluded that dietary protein, although beneficial for BMD, does not necessarily aid in the prevention of fragility fractures (Darling et al., 2009).

Meat, one of the dietary sources highest in protein, also contains high amounts of phosphorus. Phosphorus is a constituent part of crystalline hydroxyapatite, the bone mineral crystals, and is necessary in the body for correct bone mineralisation (Tanaka et al., 1988, Zhang et al., 2011). It has been demonstrated that phosphorus supplementation in conjunction with calcium stimulates bone mineralisation (Chang et al., 2000, Pohlandt, 1994), although phosphorus supplementation alone does not appear to benefit bone health (Hulley et al., 1971, Laflamme and Jowsey, 1972, Goldsmith et al., 1976). In contrast to the findings in children, it has been found that high phosphorous intake in postmenopausal women increases the incidence of mortality,

demonstrating that, as with calcium supplementation, the use of phosphorus supplementation should also be treated with great caution (Bates et al., 2012).

Meat is also one of the highest dietary sources of creatine. There is some evidence that creatine monohydrate supplementation may help with the effects of muscular dystrophy, and subsequent low bone mass in children (Tarnopolsky et al., 2004b). Creatine monohydrate supplementation in adult rats results in increased BMD and bone strength (Antolic et al., 2007), increased osteoblast activity and mineralisation in vivo (Gerber et al., 2005). Furthermore, creatine monohydrate supplementation in older adults can result in increases in bone mineral content (Chilibeck et al., 2005) and decreases in bone resorption (Candow et al., 2008). Despite some disagreement in the literature (Gualano et al., 2014a, Lobo et al., 2015), it has therefore been suggested that creatine supplementation may have potential therapeutic effects for treatment of bone disorders (Gualano et al., 2010, Gualano et al., 2016) (see 2.6.7).

2.6 Creatine for Muscle and Bone Health

Dietary supplementation with creatine has been shown to increase gains in lean mass and muscle strength, and when combined with exercise, to result in gains beyond those from exercise alone (Camic et al., 2014, Earnest et al., 1995, Kreider et al., 1998a, Kreider, 2003, van Loon et al., 2003). Given the relationship between muscle and bone (see 2.2), increased muscle mass and strength following creatine supplementation may result in adaptations in the bone. Creatine supplementation may also have a direct effect on bone, through stimulating osteoblast activity (Gerber et al., 2005, Gerber et al., 2008). Dietary supplementation with creatine in rodent models of osteoporosis, and in some forms of muscular dystrophy, has shown a beneficial effect on markers of bone turnover, BMD, and bone strength (Antolic et al., 2007, Louis et al., 2002, Tarnopolsky et al., 2004b).

2.6.1 Creatine synthesis

Creatine, methylguanidine-acetate acid, is a nitrogenous organic acid which is required in the human body for energy production. Creatine can be obtained from the diet, where it is prevalent in red meat and fish, and other meat sources. Creatine can be synthesised in the body, in the liver and kidneys, from the amino acids arginine, glycine, and

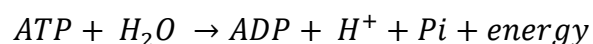
methionine. Arginine and glycine react together to form guanadinoacetate (GAA), which then reacts with methionine to form methylguanidine-acetate acid. Creatine synthesis is inhibited by intake, meaning that individuals who have a lower creatine intake have a higher rate of endogenous synthesis (Derave et al., 2004). Daily turnover of creatine results in the waste product creatinine; creatinine is excreted in the urine, as is excess dietary creatine. A decrease in dietary creatine intake results in a decrease in urinary excretion of creatine and creatinine (Bleiler and Schedl, 1962, Crim et al., 1976, Derave et al., 2004), suggesting that endogenous synthesis may not result in as high creatine levels within the body's muscle stores as creatine intake, although a decrease in creatine retention following exogenous creatine supplementation has been suggested (Derave et al., 2004).

Approximately 95% of the body's creatine stores are in the muscle; skeletal, smooth, and cardiac. The remaining 5% is stored in the brain, testes, liver, and kidneys. Creatine uptake into the muscle is controlled by a sodium-dependent saturable active transport process, and is phosphorylated in the muscle, where it is stored as phosphocreatine (PCr). The concentration of creatine stored in muscle is dependent on the muscle; type II muscle fibres store approximately 20% more creatine than type I fibres, which is associated with the higher glycolytic capacity of type II muscle fibres (Soderlund and Hultman, 1991).

2.6.2 Energy Production

Energy for muscle contraction is produced by the hydrolysis of adenosine triphosphate (ATP). The enzyme myosin ATPase acts to breakdown ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi).

Equation 1: Breakdown of ATP to release energy



Muscle contraction begins when ATP and calcium are both present in the muscle cell in sufficient quantities. The sarcoplasmic reticulum of the muscle actively takes up calcium ions, a process which also requires ATP. In addition to this, the sodium pump requires ATP in order to restore the membrane potential of the muscle cell (the

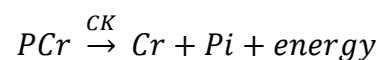
difference in electrical potential between the interior and the exterior of the cell, which allows signals to be passed between different parts of the cell).

At rest, the concentration of ATP in the muscle is approximately 4 to 5 mmol·kg w.w.⁻¹, which is sufficient for only a few seconds of high intensity exercise. ATP levels therefore need to be resynthesised in order to maintain physical activity levels. Muscle fatigue sets in when the concentration of ATP in the muscle falls by 30%; during a 30 second sprint, approximately 45% of the muscular ATP is broken down (Cheetham et al., 1986), demonstrating the importance of restoring ATP concentration levels.

2.6.3 ATP Resynthesis

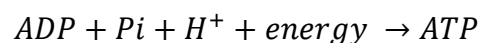
Adenosine triphosphate (ATP) is resynthesised through anaerobic metabolism. In the muscle, PCr concentrations greatly exceed ATP concentration. Phosphocreatine is broken down using the catalyst enzyme creatine kinase (CK), producing free creatine and phosphate, and releasing large amounts of energy.

Equation 2: Breakdown of PCr



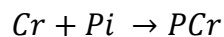
Because PCr has a higher free energy of hydrolysis than ATP, the phosphate released from the breakdown of PCr combines with ADP to synthesis ATP. This process also allows a buffer against the hydrogen ions (H⁺) which are formed during ATP hydrolysis. Given that acidification of the muscle can cause muscle fatigue, this buffer may help to delay muscle failure.

Equation 3: ATP Resynthesis



A decrease in ATP concentrations during exercise leads to PCr breakdown, releasing energy to rebuild the ATP concentration. Undergoing very intense exercise can deplete PCr levels. However, these reactions are reversible, so that during times of high energy availability, such as oxidative phosphorylation (e.g. at rest), creatine and phosphate combine to restore the muscle's PCr levels.

Equation 4: PCr Resynthesis



Muscle PCr is readily available at the onset of exercise. It allows the body to resynthesise ATP quickly, and subsequently the muscle to produce high amounts of energy. Due to limited amounts of PCr in the muscle, it can only maintain ATP levels for a short amount of time, only a few seconds of high-intensity exercise. However, this allows time for other energy systems, such as glycolysis, to reach the level of energy production necessary for the maintenance of performance.

2.6.4 Supplementation

There is wide intra-individual variation in baseline muscular creatine concentration levels. The reasons for this are as yet unknown, but it is generally assumed to be dependent on an individuals' habitual creatine intake due to the difference in creatine levels between endogenous synthesis and dietary intake (see 2.6.1). A greater increase in creatine concentration following supplementation is seen in individuals with lower baseline creatine concentrations (Burke et al., 2003, Greenhaff et al., 1994, Hultman et al., 1996). Supplementation results in a greater increase in muscular creatine concentration when creatine is ingested with carbohydrates (Green et al., 1996, Preen et al., 2003, Steenge et al., 1998); this is presumed to be because carbohydrate ingestion stimulates an insulin-dependent mechanism, stimulating sodium-potassium pump activity and so increasing creatine uptake into the muscle. Muscle creatine concentration greater than 180 mmol·kg d.w⁻¹ has been observed following seven days' high-dose supplementation of 5g consumed six times daily (Harris et al., 1992).

Supplementation with creatine has been shown to be safe, with no severe adverse effects found (Juhn and Tarnopolsky, 1998, Poortmans and Francaux, 2000). Creatine supplementation can result in weight gain, an average of 1kg (Inrig et al., 2007, Warber et al., 2002). This gain could be due to a variety of factors; an increase in intracellular water, stimulation of protein synthesis, or a decrease in protein breakdown. The decrease in urine production which mirrors creatine ingestion suggests that the former is the most likely reason (Hultman et al., 1996); the osmotic nature of creatine leads to an increase in the osmolarity of the cell, which results in water retention in the skeletal muscle fibres (Juhn and Tarnopolsky, 1998, Terjung et al., 2000). Over the long term

(>28 days), it is likely that some effect of the weight gain is due to the anabolic effects of creatine (Kreider et al., 1998a), but the anabolic effect would not be an important factor over the short term.

2.6.5 Creatine Mechanism

Although the exact mechanisms of creatine supplementation on muscle performance and growth are not known, there are several mechanisms which have been proposed. It has also been suggested that creatine likely acts by its “multifaceted” properties (Veggi et al., 2013). This is borne out by the diversity of the findings following creatine supplementation.

One possible mechanism of action of creatine is that an increased concentration of PCr in the muscle can maintain ATP levels during turnover for longer (Casey et al., 1996). It is also possible that the increased rate of PCr synthesis improves recovery during repeated bouts of exercise (Greenhaff et al., 1994). Another mechanism could be that an increase in the use of PCr to maintain ATP levels results in a decrease in anaerobic glycolysis, which forms lactic acid. The PCr would therefore provide a buffering effect, meaning that the acidity of the muscle would not rise as quickly; as a result, there would be a delay in muscle fatigue. Finally, creatine may act due to its anabolic properties.

2.6.6 Creatine for Muscle Health

Creatine supplementation has been found to have a beneficial effect on performance in various athletic populations, as well as in various disease states. It has been found that creatine supplementation in males resulted in an increase in muscular PCr resynthesis during recovery between repeated high intensity exercise bouts (Greenhaff et al., 1994). It has also been demonstrated that supplementation with polyethylene glycosylated creatine results in improved performance measures (Camic et al., 2014). Short-term creatine supplementation has been shown to have an anti-catabolic effect on some proteins in men, preventing the breakdown of the muscle (Parise, 2001). It has been shown that creatine supplementation results in a decrease in skeletal muscle content of reactive oxygen species (Guimaraes-Ferrerira et al., 2012) and the increase of markers of muscle damage which accompany repeated bouts of resistance exercise has been found to be attenuated by creatine supplementation (Veggi et al., 2013). Creatine

supplementation has been demonstrated to have a possible beneficial effect in sprinters by delaying the onset of the anaerobic threshold (Tyka et al., 2015), and in males with type 2 diabetes through improved ATP resynthesis following an increase in PCr stores (Casey et al., 1996). Twenty-eight days of pre-workout creatine supplementation in recreationally active males has been shown to increase strength of the lower body (Kendall et al., 2014); it has also been found that the addition of creatine supplementation in females going through off-season football training results in greater increases in muscle strength (Larson-Meyer et al., 2000).

However, there is some variation in the literature. Supplementation of beta-alanine along with creatine in active women has been shown not to result in any significant changes (Kresta et al., 2014), and supplementation of betaine alongside does not affect muscle strength or performance, but creatine supplementation increases muscle stores of creatine and phosphocreatine (del Favero et al., 2012). Lower limb muscle carnitine concentrations in patients suffering from type 2 diabetes appear to be unaffected by creatine supplementation (Gualano et al., 2011). 5 days of creatine loading was shown to have no significant effects on muscular fatigue during isometric force production (Smith-Ryan et al., 2014).

Creatine supplementation taken alongside corticosteroids slows the weight gain associated with steroid intake, and results in increased type 2 muscle fibre area, suggesting that creatine supplementation attenuated gain of fat mass, while stimulating growth of lean mass (Roy et al., 2002). It has been demonstrated that creatine supplementation slows the impaired running of hamsters which are fed with corticosteroids (Campos et al., 2006). In addition to this, it has been shown that creatine supplementation along with dexamethasone (DMS) attenuates the decreased maximal exercise duration and oxygen consumption associated with DMS (Menezes et al., 2007). It was also found that the attenuation of gastrocnemius and diaphragm muscle weight loss, and the atrophy of type 2 fibres in the gastrocnemius associated with DMS were attenuated by creatine supplementation (Menenez et al., 2007). It has therefore been suggested that creatine supplementation may be useful in the treatment of steroid-induced myopathy (Menenez et al., 2007). This demonstrates that oral supplementation with creatine may be used to slow the catabolic effect of corticosteroids; it decreases

loss of muscle strength and function, mirroring its effects on preventing breakdown of muscle in healthy men (Parise, 2001).

Short- and medium-term creatine supplementation have been shown to increase muscle strength and improve functional performance in male and female patients suffering from particular muscular dystrophies (Kley et al., 2013), and can delay the degeneration of some muscular disorders (Gualano et al., 2010). Creatine supplementation in patients suffering from neuromuscular disease has been shown to increase body weight as well as strength measures (Tarnopolsky and Martin, 1999). Previous findings have demonstrated that creatine supplementation in patients suffering from muscular dystrophy improved maximal voluntary contraction strength and resistance to fatigue (Louis et al., 2002). However, in patients suffering from myotonic dystrophy type 1, creatine supplementation does not appear to affect body composition, or muscular strength or activities of daily living (Tarnopolsky et al., 2004a). In addition to this, high dose creatine supplementation in patients suffering from McArdle disease has been found to result in impaired activities of daily living and increased muscle pain (Kley et al., 2013).

The addition of creatine supplementation in males already undergoing resistance or agility training can result in greater gains in lean mass as well as performance (Kreider et al., 1998a). Creatine supplementation alongside resistance exercise in men has been shown to result in increases in fat-free mass (FFM) and in body weight as well as in performance measures (Camic et al., 2014, Earnest et al., 1995, van Loon et al., 2003). However, it has been demonstrated that creatine supplementation in rats caused insulin resistance, and did not cause attenuation of muscle wastage (Nicastro et al., 2012). Despite an increase in muscle strength, female footballers undergoing off-season training did not show significant increases in lean mass following creatine supplementation (Larson-Meyer et al., 2000); off-season training may not have been of a high enough intensity to elicit the muscle growth associated with supplementation.

Creatine supplementation has a greater effect on muscle PCr availability in older men and women than in younger (Rawson et al., 2002, Smith et al., 1998). However, it has been found that there is no difference in blood or urine creatine levels between groups (Rawson et al., 2002). Furthermore, recent reviews have demonstrated that creatine supplementation may benefit elderly individuals and some patient populations (Gualano

et al., 2012), and that creatine supplementation in elderly individuals without training results in attenuation of the loss of muscle mass and performance (Moon et al., 2013). Seven days of creatine supplementation has been shown to increase performance as well as attenuating the loss of muscle strength, and improve functional fitness, in older men (Gotshalk, 2002). Creatine supplementation has therefore been suggested as a possible therapeutic tool in elderly individuals (Gotshalk, 2002, Gualano et al., 2010, Gualano et al., 2012, Moon et al., 2013).

The effects of various creatine doses on muscle strength and performance have been investigated. High-dose supplementation over the short-term ($>10\text{g}\cdot\text{day}^{-1}$, <7 days) results in increased fat free mass and body mass in men and women (Mihic, 2000), although it is hypothesised that this may be partially due to fluid retention (see 2.6.4). Supplementation of $20\text{g}\cdot\text{day}^{-1}$ for 5-6 days can also significantly improve exercise performance (Greenhaff et al., 1993, Greenhaff et al., 1994, Hultman et al., 1996). Subsequent studies investigated the effects of a “loading dose” of up to $20\text{g}\cdot\text{day}^{-1}$ followed by a low dose “maintenance phase”, which results in sustained increases in performance, strength, and muscular creatine concentration (Chung et al., 2007, Hultman et al., 1996, Preen et al., 2003, Tarnopolsky et al., 1997). Long-term, low doses (>28 days, $<7\text{g}\cdot\text{day}^{-1}$) result in increases in lean mass, fat free mass, and muscle function (Aguiar et al., 2013, Candow et al., 2015, Chilibeck et al., 2014, Gualano et al., 2014b, Wilkinson et al., 2015). It has been demonstrated that supplementation of $3\text{g}\cdot\text{day}^{-1}$ for 28 days results in the same increase in muscular creatine stores as a loading phase of $20\text{g}\cdot\text{day}^{-1}$ for 6 days followed by a maintenance phase of $2\text{g}\cdot\text{day}^{-1}$ (Hultman et al., 1996). There is increasing interest in the effects of creatine supplementation on markers of bone health, particularly in populations which are at risk for bone disorders. Supplementation of $1\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ (average dose $5.7\text{g}\cdot\text{day}^{-1}$) during a resistance training program increased BMD and measures of bone strength in postmenopausal women (Chilibeck et al., 2014), although a loading phase of $20\text{g}\cdot\text{day}^{-1}$ for 5 days, followed by $5\text{g}\cdot\text{day}^{-1}$ during resistance training, did not result in changes in markers of bone health in postmenopausal women already diagnosed with osteopenia or osteoporosis (Gualano et al., 2014b).

2.6.7 Creatine and Bone Health

According to Wolff's Law and the subsequently described muscle-bone unit (see 2.2), an increase in muscle mass, such as that following creatine supplementation, should result in an increased pull of the muscle on the bone, and therefore an increase in bone stress and so a subsequent increase in bone formation (Frost, 1994, Ruff et al., 2006, Schoenau, 2005). Creatine supplementation may have a more direct effect on bone, by stimulating the activity and mineralisation of osteoblast-like cells, and by causing chondrocyte hypertrophy (Antolic et al., 2007, Gerber et al., 2005).

It has been demonstrated that creatine supplementation in boys suffering from muscular dystrophy resulted in a decrease in markers of bone breakdown (Louis et al., 2002, Tarnopolsky et al., 2004b). Creatine supplementation may also result in attenuation of degradation in disease states exhibiting bone and metabolic disturbances (Gualano et al., 2010). Findings have demonstrated that creatine supplementation has a beneficial effect on BMD and bone strength in rats (Antolic et al., 2007), and that an increase in the phosphate content of trabecular bone follows creatine supplementation in ovariectomised rats, suggesting that there may be beneficial effect of creatine supplementation on chemical composition of bone following ovariectomy (de Souza et al., 2012). However, these results have been contradicted by recent findings that exercise, but not creatine supplementation, attenuated bone loss in ovariectomised rats (Murai et al., 2015). It has also been demonstrated that creatine supplementation in spontaneously hypertensive rats, an experimental model for osteoporosis, did not result in significant changes in bone mass (Alves et al., 2012); however, this study investigated only male rats, and the gender differences in bone responses is well documented. It should also be noted that creatine metabolism seems to vary widely between species, and so the results of studies conducted on non-humans may not be directly transferable.

As a result of increasing the activity of osteoblast-like cells (Gerber et al., 2005), creatine also stimulates the release of OPG, inhibiting osteoclast activity (Yasuda et al., 1998) (see 2.1.3). This results in a reduction in markers of bone resorption (Candow et al., 2008, Cornish et al., 2009). Given the predominance of osteoclast activity in bone turnover in older individuals, it has been concluded that creatine supplementation may

be beneficial for BMD and markers of bone health in this population (Antolic et al., 2007, Candow and Chilibeck, 2010, Candow et al., 2014a).

2.7 Creatine and Exercise

2.7.1 Creatine and Exercise Effects on Muscle Health

Creatine supplementation in combination with exercise has been shown to result in increased muscle mass and strength (see 2.6.6). Creatine supplementation during strength and power exercise results in greater increases in muscle mass and strength than exercise alone, possibly due to the increased PCr stores allowing greater training stimulus and consequently a greater effect of training (Casey et al., 1996, Greenhaff et al., 1993). However, creatine supplementation has not been shown to affect aerobic or endurance exercise performance (Balsom et al., 1993, Thompson et al., 1996, Vanakoski et al., 1998). Exercise in combination with creatine supplementation results in improved athletic performance in strength and power activities, and is associated with increased lean mass and decreased fat mass in comparison to exercise alone in men and women (Brose et al., 2003, Earnest et al., 1995, Kirksey et al., 1999).

Creatine supplementation during resistance training has been shown to result in increases in muscle mass and strength in older women (Gualano et al., 2014a). 12 weeks of resistance training with creatine supplementation results in increases in muscle mass, FFM as measured by DXA, and muscle strength in older women (Aguiar et al., 2013, Tarnopolsky et al., 2007). The timing of creatine ingestion in relation to exercise does not affect the results of supplementation on muscle (Candow et al., 2014b). It has also been shown that creatine supplementation during 8 weeks of resistance training resulted in increases in IGF-1, possibly a factor in the muscle hypertrophy seen following creatine supplementation (Burke et al., 2008).

In contrast to these findings, creatine supplementation in combination with resistance training in rats has not been shown to result in muscle mass gains above training alone (Aguiar et al., 2011). Supplementation with creatine along with resistance training in healthy men and in male cardiac patients has been shown to result in no additional gains above training alone (Bemben et al., 2010, Cooke et al., 2014, Cornelissen et al., 2010). However, none of these studies investigated creatine supplementation in women, therefore that the findings are not conclusive in terms of adult women. It has also been

demonstrated that experienced, trained women did not receive additional benefits from supplementation with creatine, although it was hypothesised that this may be due to non-responders (Ferguson and Syrotuik, 2006); women in this study were already trained, and so the exercise they underwent did not load the body more than habitual activity. Meta-analyses have concluded that creatine supplementation in combination with resistance training can promote healthy aging in both men and women through beneficial effects on muscle mass and strength, and function (Candow et al., 2014a, Devries and Phillip, 2014).

2.7.2 Creatine and Exercise Effects on Bone Health

Bone is a dynamic tissue, which is constantly being remodelled. Osteoblast-like cells have been shown to have increased differentiation and mineralisation following creatine supplementation (Gerber et al., 2005), and osteopenic osteoblast cells have been shown to have increased levels of osteoprotegerin secretion following creatine supplementation (Gerber et al., 2008). Ovariectomised rats undergoing 8 weeks of creatine monohydrate supplementation show increases in BMD and bone strength (Antolic et al., 2007). There are however some studies which disagree with these findings; it has been found that 12 weeks of creatine supplementation does not influence bone loss in ovariectomised rats (Murai et al., 2015).

Creatine supplementation in boys suffering from muscular dystrophy has been shown to have beneficial effects on measures of bone health after 3-4 months (Louis et al., 2002, Tarnopolsky et al., 2004b). Although Louis et al (Louis et al., 2002) did not find BMD changes in immobile patients, those who were able to walk had significant increases in BMD, suggesting that the combination of physical activity and creatine supplementation was responsible for these changes.

Supplementation of creatine monohydrate during 12 weeks of resistance training in older males results in increases in upper-limb BMC and BMD (Chilibeck et al., 2005). It has been demonstrated that 10 weeks of creatine supplementation during resistance training resulted in decreases in markers of bone resorption in older men (Candow et al., 2008). It has also been concluded that creatine supplementation during resistance training results in additional increases in BMD over training alone in older adults (Candow and Chilibeck, 2010). In addition, 52 weeks of resistance training in

combination with creatine supplementation has been found to result in attenuation of bone loss in the proximal femur in postmenopausal women (Chilibeck et al., 2014).

Increases in BMD following creatine supplementation in combination with exercise may be an indirect effect attributable to the muscle (Chilibeck et al., 2005). It is also possible that the effects on bone are due to effects of creatine supplementation on osteoblasts (Gerber et al., 2005, Gerber et al., 2008); markers of bone resorption are decreased following supplementation (Candow et al., 2008, Cornish et al., 2009), suggesting a direct effect of creatine on the cells involved in bone turnover. Osteoblasts receive energy from creatine kinase (CK); increases in PCr result in increases in CK, and so increased energy for the bone-forming cells.

However, it has been found that 24 weeks of creatine supplementation in combination with resistance training did not result in increases in bone mass in older women (Gualano et al., 2014a). It has also been demonstrated that 4 weeks creatine monohydrate supplementation in addition to resistance training does not result in additional changes in BMD in young trained males (Volek et al., 2004, Kreider et al., 1998b). However, these studies do not use additional training above the participants' current daily activity levels, and so would not cause as much stress on the bone as training interventions which use additive training levels. It has further been shown that there is no additive effect of creatine supplementation on bone during 14 weeks of resistance training (Brose et al., 2003), or during 6 months of resistance training (Tarnopolsky et al., 2007). It has been concluded that creatine monohydrate supplementation may have a future therapeutic role in bone health, but that more research needs to be done to qualify the therapeutic effects (Candow et al., 2014a, Gualano et al., 2010, Gualano et al., 2016).

In conclusion, there is a potential for creatine supplementation to have a beneficial effect on both muscle and bone in ageing women. The effect of creatine on bone could be a direct effect, or could be an indirect effect mediated by the muscle (Candow et al., 2014a, Chilibeck et al., 2005). Effects of creatine supplementation on both muscle and bone are likely to be augmented by the addition of exercise (Candow et al., 2014a, Gualano et al., 2014b, Louis et al., 2002). The role of creatine, and the possible beneficial effects of creatine supplementation, in the bone health of premenopausal

adult women, a population at risk for the future development of bone degenerative disorders, remains to be investigated.

2.8 Literature Review Summary

Bone turnover and remodelling is a process which occurs continually throughout life. During early life, bone formation occurs at a greater rate than bone resorption, and so bone mass accrual occurs. This continues until the mid-twenties, when the next phase begins. During early adult life, bone mass is maintained through equal bone formation and resorption. As individuals age, bone resorption begins to exceed bone formation, and so bone loss occurs. In women, the change of hormones which occurs during the menopause, the decrease in oestrogen, means that bone loss can occur at a high rate. Osteoporosis, a disease state which is defined by low bone density, is therefore more prevalent in women than in men. Osteoporosis is more likely in individuals who have a high rate of bone loss, or who do not reach a high peak bone density before bone loss occurs. Increasing and maintaining bone density during adult life therefore decreases the risk of developing osteoporosis and osteoporosis-related disorders in later life.

Wolff's Law states that bones adapt to stresses placed upon them. Physical activity during early life results in greater peak bone density during adulthood. Physically active adults exhibit higher bone density and greater markers of bone formation than sedentary individuals, and physical activity in older individuals results in truncated bone loss. Physically active individuals therefore have a decreased risk of developing osteoporosis in later life, due to higher peak bone density, increased bone formation, and decreased bone resorption. Physical activity also increases muscle mass, which is necessary for maintenance of independence, mobility, and quality of life in older individuals. Muscle mass is correlated with bone density, although it is unclear which stimulates which, or whether both muscle and bone growth are stimulated by a third factor.

Creatine supplementation has been suggested to have beneficial effects on bone density and bone formation markers in *in vitro* and *in vivo* studies. Creatine supplementation results in increased muscle mass and muscle strength, which in turn increases the pull of muscle on bone and so may result in subsequent bone adaptations. Creatine supplementation also allows more high intensity work to be completed before fatigue

sets in, therefore increasing the amount of work completed and so increasing the impact on the bone, potentially stimulating greater bone adaptation.

In an ageing but inactive population, the need for improving musculoskeletal health is increasing. Given the high prevalence of osteoporosis and osteopenia in postmenopausal women, and the associated health and economic costs, interventions which are preventative rather than curative in nature and which decrease risk factors for future development of low BMD are increasingly important. Premenopausal women are at risk for developing low BMD in the future, and so decreasing risk factors in premenopausal women could result in reduced incidence of osteoporosis in the future. Exercise is an economic and generally accessible intervention, and creatine is a low-cost supplement with no adverse side-effects. Although studies are emerging which investigate the effects of creatine supplementation and exercise on musculoskeletal health, these studies investigate populations which already have low BMD, and so are curative in nature, rather than preventative. This thesis therefore aims to investigate the effects of creatine supplementation, and the effects of creatine supplementation and exercise, on musculoskeletal health in premenopausal adult women.

2.9 Aims and Objectives

2.9.1 Aims

This thesis therefore sets out to investigate the effects of exercise and creatine supplementation on musculoskeletal health in premenopausal women. This will be achieved through the following aims;

1. Establish the reliability of the current institution's DXA scanner
2. Determine the reliability of DXA measurements of body composition during a period of creatine supplementation
3. Explore the relationships between physical activity and BMD, lean mass, and muscle strength in premenopausal women
4. Examine the relationship between creatine intake and BMD, bone turnover, lean mass, and muscle strength in premenopausal women
5. Investigate the effects of creatine supplementation and exercise on BMD, bone turnover, lean mass, and muscle strength in premenopausal women

Experimental studies carried out in this thesis use a population of adult women. For the purposes of this thesis, this population is defined as women who have reached peak bone mass, and are in the bone consolidation phase, before the bone loss associated with the menopause occurs; participants range in age from 30 years to 60 years old.

2.9.2 Objectives

This thesis sets out to achieve these aims through the following objectives;

1. Complete a precision study in line with recommendations to determine the reliability of the present institution's DXA scanner, and to quantify the error of the machine and the operator.
2. Perform an exploratory study to investigate the effects of creatine supplementation on hydration status and DXA measure of BMD, fat-free mass, and fat mass.
3. Develop an understanding of the underlying relationships between physical activity, creatine intake, bone density, fat-free mass, and functional muscle strength in adult women.

4. Determine the influence of an oral creatine supplementation regime on bone health, fat -free mass, and muscle strength in adult women
5. Design and evaluate an intervention aiming to investigate the effects of high impact exercise augmented by creatine on musculoskeletal health in adult women

3 General Methods

This chapter gives a brief overview of each study design. Thorough details of the methods used throughout the experimental studies detailed in this thesis are then described. Brief descriptions of methods used are given in each experimental chapter.

3.1 Study Designs

In completion of this thesis, five experimental studies were conducted. The two initial studies, described in section 1, were precision studies, designed to validate the measures of BMD and body composition by DXA, the main measure used throughout the thesis. Study 1 was designed to be a precision study, investigating the reliability of the current institution's DXA in accurately measuring change over time. Study 2, which was a double-blind, randomised control study, was then designed to assess the effects of creatine supplementation on DXA measures of BMD and body composition, to assess whether DXA could be used to accurately assess body composition in a supplementing population, or the supplement caused body composition changes which introduced error into the DXA measurements.

Once the DXA measures had been validated, study 3, which was a cohort study, investigated the relationships between habitual creatine intake, BMD, muscle mass, and muscle strength in adult women. Building on the findings regarding habitual creatine intake, the subsequent study then increased creatine intake through use of an oral supplement. Study 4 was a double-blind, randomised control trial which investigated the effects of long-term, low-dose creatine supplementation, at doses greater than those consumed habitually through the normal diet, on bone turnover, BMD, and muscle mass and strength. These early experimental studies then lead onto the design for study 5, which built on the earlier studies. The inclusion of exercise alongside the creatine supplementation was intended to increase the uptake of creatine into the working muscle. At the same time, creatine supplementation was anticipated to improve exercise performance by allowing more high intensity work to be completed, thereby increasing the training adaptations. Study 5 was a double-blind, randomised control trial; it assessed the effects of an exercise program, and exercise augmented by oral creatine supplementation, on bone turnover, BMD, and muscle mass and strength. Studies 3, 4, and 5 are described in section 2 of the present thesis.

3.2 Participants

Studies 1, 3, 4, and 5 (Chapters 4, 6, 7, and 8, respectively) use adult female participants. Participants were aged 30-60 (45.0 ± 12.1 years), height (165.6 ± 6.2 cm), weight (70.4 ± 13.2 kg), BMI (25.6 ± 4.5 kg·m⁻²). Study 2 used both male and female participants (male=22, female=14). Participants were (20.5 ± 2.0 years) height (174.7 ± 10.1 cm), weight (77.0 ± 16.8 kg), BMI (25.3 ± 5.7 kg·m⁻²).

3.2.1 Recruitment

Participants were recruited via a number of methods. Adverts were placed in the university bulletin, and posters placed around the university Arts Centre. Information regarding the studies was also circulated around the nearby council and government buildings. Word of mouth was the most effective method of circulating information and contact details regarding the study.

3.2.2 Inclusion/exclusion

For studies investigating women only, inclusion criteria was women over the age of 30. For study 2, investigating men and women, inclusion criteria was individuals over the age of 18.

Exclusion criteria were; those currently undergoing treatment for osteoporosis, those who had taken a creatine supplement in the 3 months previous, anyone who was or might be pregnant, anyone with renal function problems, those with large metal implants (e.g. hip replacement), and individuals habitually exceeding than the current government guidelines for daily physical activity levels.

3.2.3 Ethical clearance

Prior to any testing being conducted, ethical approval was granted for each study by Aberystwyth University, in accordance with the Ethical Guidelines of the Helsinki Declaration of 1961 (revised in Fortaleza, 2013).

3.2.4 Informed consent procedure

When participants initially expressed interest in taking part in studies, they were given a participant information sheet to read through. If after reading the information sheet

individuals were willing to take part in the study, they were invited to attend the laboratory. On arrival at the laboratory, they were again given a copy of the information sheet, and were given the opportunity to answer any questions they may have had about their involvement. Individuals who were then happy to take part in the study were asked to sign informed consent forms prior to any testing being conducted.

All participant information sheets and informed consent forms can be found in the Appendices.

3.2.5 Confounding Factors

In each experimental trial, habitual creatine intake, calcium intake, and habitual physical activity levels were assessed at the beginning of and during each trial, and were controlled for. A menstrual health questionnaire (Appendix) was completed by each participant at the beginning of each experimental trial. This included questions on use of hormonal contraceptives such as the oral contraceptive pill, the number of previous pregnancies individuals had experienced, and the number of menstrual cycles individuals had experienced in the 12 months prior. This allowed assessment of menstrual status, and for use of hormonal contraceptives to be controlled for. No participants were classed as experiencing amenorrhea or oligoamenorrhea at the onset of each trial. Although the female athlete triad syndrome is exhibited in non-athletes as well as in athletes (Torstveit and Sundgot-Borgen, 2005a), no participants exhibited amenorrhea, suggesting that participants in the present thesis did not suffer from the syndrome.

3.3 DXA

There are multiple methods of assessing body composition, such as bioelectrical impedance analysis (BIA), hydrostatic weighing, BMI, skinfold measures, and air displacement plethysmography. Dual-energy X-ray absorptiometry (DXA) is a body composition assessment tool which can measure BMD and BMC in addition to fat mass and lean mass. Hydrostatic weighing has been previously used as a ‘gold standard’ method of measuring body composition (De Lorenzo et al., 1997), however DXA has been suggested to be a better method of assessing body composition in older adults, as it can account for age-related decreases in BMD (Brodowicz et al., 1994). DXA has

been shown to be a more reliable method of body composition assessment than air displacement plethysmography (Lowry and Tomiyama, 2015), and is subject to fewer violable assumptions than BIA, BMI, skinfold measures, such as muscle-to-fat ratio, fat content of adipose tissue, and internal and subcutaneous fat deposition (Bedogni et al., 2001, Martin et al., 1985, Wells and Fewtrell, 2006). Due to its safety and ease of use, DXA is widely recognised as the ‘gold standard’ method of assessing body composition (Bosaeus et al., 1996, Schnabel et al., 2005, Toombs et al., 2012).

3.3.1 Protocol

Dual-energy X-ray absorptiometry (DXA) scans were performed using a Hologic Discovery A QDR (APEX System Software Version 3.4.2). The participant completed a medical questionnaire (see Appendix D. DXA radiation exposure questionnaire) to ensure that they met the Institutional ethical requirements for undergoing a DXA scan and that they were within the annual radiation limits of the UK Government (Health and Safety Executive). They were then asked to wear only lightweight clothing, and to remove any items containing metal (e.g. underwire bra). Hospital scrubs, consisting of lightweight trousers and tunic, were provided as alternative clothing if necessary. The participant then lay in the supine position on the scan table. A whole body scan, a lumbar spine scan, and a proximal femur scan were then performed, respectively.

3.3.2 DXA Calibration

Quality assurance of the DXA was performed in accordance with the manufacturers’ standards. Daily calibration and quality control were performed using the manufacturer supplied lumbar spine phantom, and weekly radiographic uniformity was performed, to ensure that the calibration of the densitometer was regularly monitored. The DXA also underwent regular full servicing to ensure that the calibration values were remaining normal. The CV for daily calibration over the past 12 months is 0.20%. DXA calibration graphs are presented in Appendix A.

3.3.3 Scan Procedure

DXA scans on the Hologic QDR Discovery A densitometer can be performed in a number of modes; express, array, and high definition. Scan modes vary in terms of spatial resolution, scan time, and radiation dose. Although there are no standardised

rules guidelines regarding the use of scan modes, it has been shown that scans using different modes on a Hologic Discovery QDR A densitometer show excellent intra-scan mode reproducibility, but it is suggested that repeated scans are performed using a single mode to limit sources of error (Bandirali et al., 2014, Messina et al., 2013). Furthermore, faster scans have been shown to be generally preferable due to the speed and low radiation dose, except in specific circumstances such as obesity, where a slower scan with higher resolution may be preferable (Bandirali et al., 2014, Messina et al., 2013). All scans were performed in the default scan mode, as set by the manufacturer.

3.3.3.1 *Whole-body*

For the whole body scan the participant lay centrally on the table in the supine position, ensuring that they lay entirely within the scan area. Any participant who was too tall for the bed was moved towards the head end so that their feet were within the scan area, as the head was excluded from the analysis. No subjects were too wide for the scan area. The legs were gently pulled and the shoulders pushed towards the feet to ensure that the pelvic and shoulder girdles aligned. The legs were kept straight and relaxed. The arms were kept straight next to the sides, and low density foam pads were placed between the hands and the hips to enable analysis (Figure 3.1).



Figure 3.1: Participant positioning for whole body scans

3.3.3.2 L1-L4 Lumbar Spine

For the lumbar spine scan, the participant initially lay supine centrally on the table. The legs were then raised, and the large square knee positioner was placed underneath the lower leg, with the cushion height adjusted to keep the lower leg parallel to the table (Figure 3.2). The participant was asked to rest their arms by their sides. The participant's iliac crest was then located, and the scan arm was adjusted so that the horizontal line of the cross-hair (in relation to the patient) was approximately in line with the iliac crest. The vertical line of the laser cross hair lay along the midline of the participant.

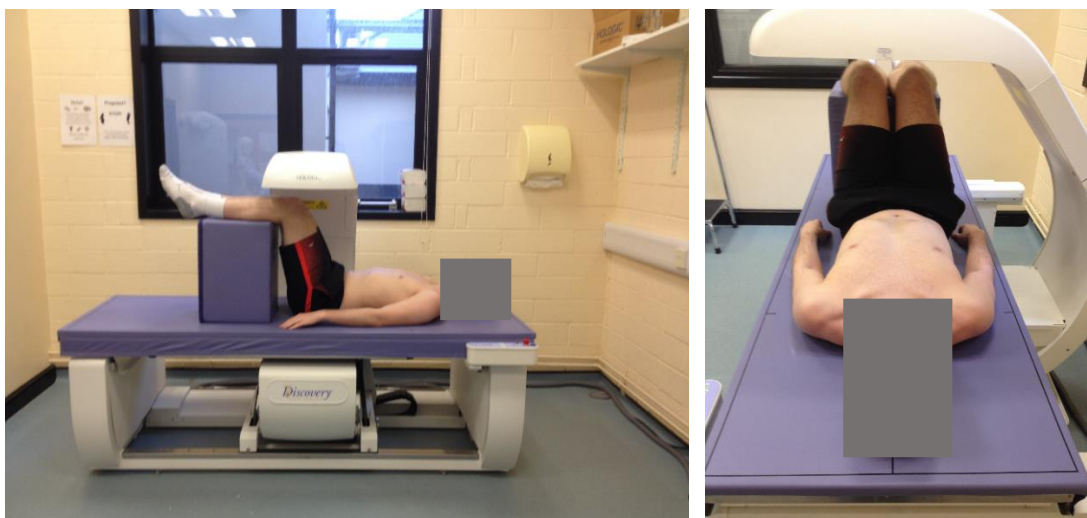


Figure 3.2: Participant positioning for lumbar spine scans

3.3.3.3 Proximal Femur

The participant lay in the supine position centred on the scanner table. The hip scan positioning fixture was placed centrally between the participant's feet, and the left leg was rotated internally so that the foot could be secured against the positioning fixture (Figure 3.3). The leg was then abducted to ensure that the femur was parallel to the long axis of the table. Once the leg was in place the greater trochanter of the left hip was located and the scan arm was adjusted so that the laser cross-hair was over the greater trochanter.

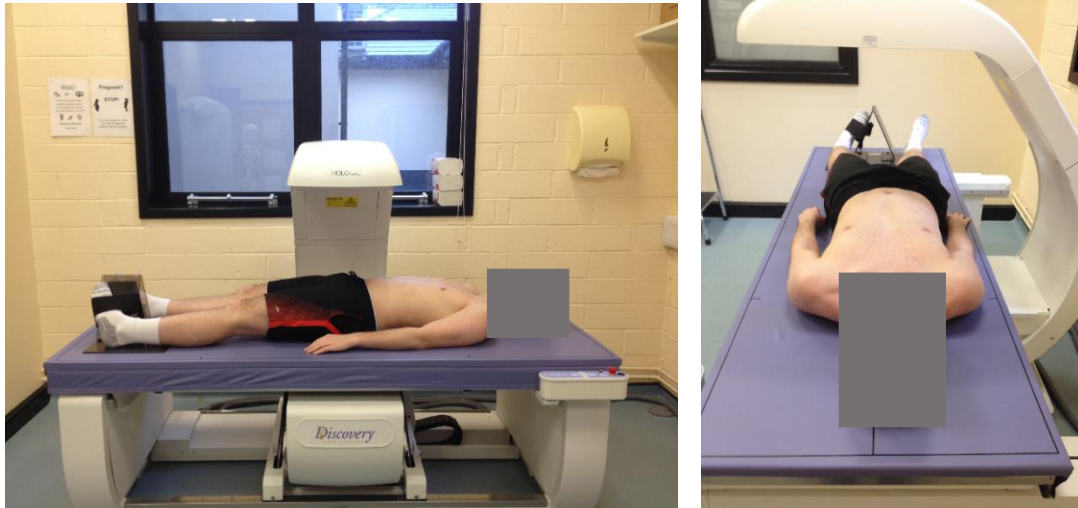


Figure 3.3: Participant positioning for proximal femur scans

3.3.4 Scan Analysis

After all scans for all participants has been completed, they were analysed by the investigator within a 12-hour period. The automatic hip and spine scan analyses were adjusted to ensure that they were properly segmented. For the proximal femur scan, the region of interest (RoI) included the lesser trochanter, the greater trochanter and the insertion of the ball and socket joint (Figure 3.4). For the lumbar spine scan, the RoI included L1, L2, L3, and L4. The automatic segmentation lines were manually adjusted to ensure that they ran between each vertebra (Figure 3.4). Manual adjustments were made to the automatic analysis by adding or removing necessary bone pixels, or by adjusting the position of the neck box on the proximal femur scan.

The whole body scans were manually segmented into regions; head, trunk, left arm, right arm, left leg and right leg. For the purposes of analysis, the arms and legs were also combined into regions, “upper limbs” and “lower limbs”. This involved addition of the components “fat mass”, “lean mass” and “total mass”, and recalculation of the percentage fat. Statistical tests were then performed on these regions as on the other regions (Figure 3.4).

3.3.5 Scan Images

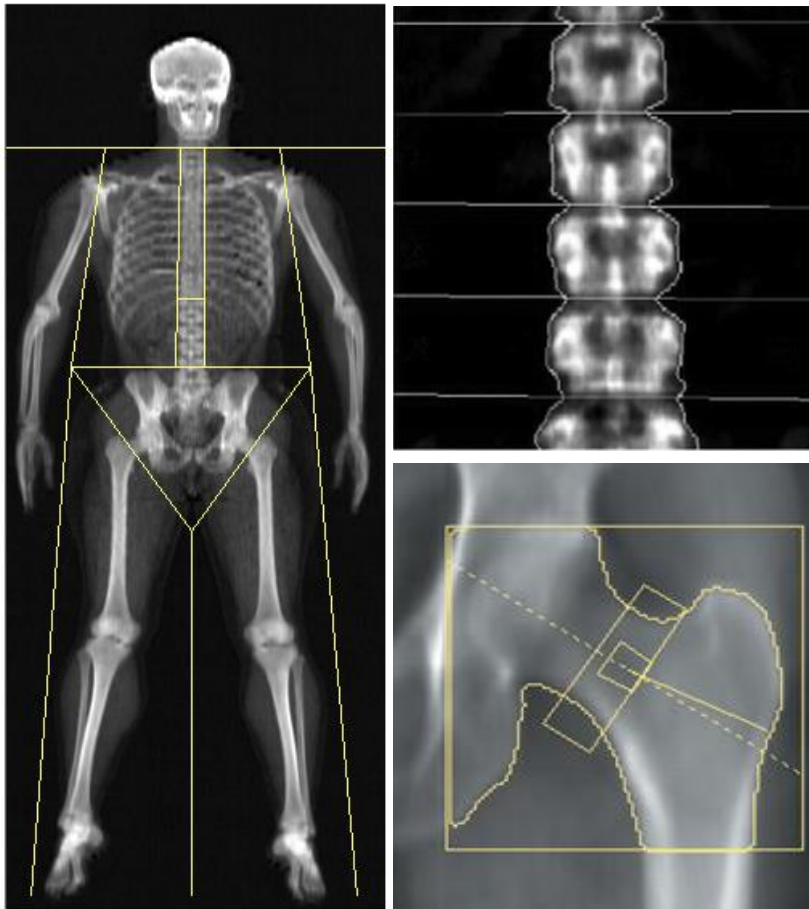


Figure 3.4: DXA scan images of the whole body (left), lumbar spine (above right), and proximal femur (below right)

3.4 Biodex

Participants completed strength tests on an isokinetic dynamometer (Biodex [Biodex Isokinetic System III, IPRS Mediquipe, Little Blakenham, UK]), to measure functional strength of the quadriceps and hamstrings of the left leg. The left leg is the standard measure used by DXA, so the left leg was used by Biodex so that the muscle strength was directly comparable to fat-free mass and BMD measured by DXA. Participants were strapped securely into the Biodex, with straps running across the chest and waist, and over the thigh of the left leg, to isolate the required muscle groups. Participants warmed up on the Biodex by performing 15 contractions and extensions of the knee without resistance. This warmup also served as a familiarisation for the trials.

The knee was held at an initial angle of 90°. Participants were asked to extend their leg as far as they could comfortably, and then to retract as far as they could comfortably. These limits were set as the range of motion, so that the knee movement would not go outside this range and risk damaging the knee. Participants performed 5 maximal extensions and contractions at 60°.sec⁻¹ angular velocity with verbal encouragement. After a 10 second rest, the test was then repeated at 120°.sec⁻¹ angular velocity, again with verbal encouragement. Peak torque was recorded at each angular velocity.

3.5 Bone Turnover Markers

There are a variety of markers of bone turnover which could have been used in the experimental studies. Given budget constraints, there was a limit to the markers which were possible to use for these studies. Serum samples were transported to Swansea University Medical School, where all blood analyses were performed. The current investigator accompanied the samples and took part in the analysis.

Procollagen type 1 propeptides can also be used as a measure of bone formation rate, although it is a more sensitive marker in an osteoporotic population than in a healthy population (Marin et al., 2011). Although alkaline phosphatase (ALP) activity arises from liver as well as from bone, in a population with no liver disease it is a reliable marker of changes in bone formation (Brown et al., 2009). Osteocalcin (OC) has good tissue specificity, and low variation, although can be sensitive to haemolysis of the blood sample (Brown et al., 2009). Studies conducted in the present thesis therefore use the combination of ALP and OC to measure bone formation.

Markers of bone resorption include hydroxyproline, hydroxypyridinium cross-links PYD and DYD, type 1 collagen C-telopeptide, C-telopeptides, and N-telopeptides. Hydroxyproline was in common use until the early 1990s, but lacks specificity and sensitivity (Simsek et al., 2004). Both PYD and DYD are more reliable measures of bone resorption in urine than in serum (Vasikaran et al., 2011). Type 1 collagen C-telopeptide is not sensitive to metabolic bone processes such as osteoporosis and rapidly loses signal, and C-telopeptides have large diurnal variation, making them unreliable for use when samples may not be taken at the same time each day (Vasikaran et al., 2011, Wichers et al., 1999). N-telopeptides (NTX) are correlated with increased bone turnover and show similar stability to C-telopeptides, and although it is a less

sensitive marker than C-telopeptides, it also shows less diurnal variation (Stokes et al., 2011, Vasikaran et al., 2011). NTX is also a more economically viable test. Studies conducted in the present thesis therefore use NTX as a measure of bone resorption. The ALP:NTX ratio is used as a measure of bone turnover by comparing the rate of bone formation to bone resorption.

Both ALP and NTX are stable when samples are stored at -20°C or below, but OC requires storage at cooler temperatures to prevent sample instability. All samples were stored at -80°C, which is sufficient for sample stability.

Sample analyses used have intra-analysis variation which affects the reliability of the measurement over time. The manufacturer reported CV of ALP analysis for intra-assay precision is less than 8%, and inter-assay precision is less than 10%. The manufacturer reported CV of OC analysis for intra-assay precision is 8.54%, and inter-assay precision is 3.73%. The manufacturer reported CV of NTX analysis for intra-assay precision is less than 8%, and inter-assay precision is less than 10%. All blood sample analysis was done in duplicate to ensure reliability of the measurement. If the results differed by more than 15%, the analysis was repeated (CHMP, 2012).

3.5.1 Blood Sampling

Venous blood samples were taken from each participant. All blood samples were taken by individuals trained in phlebotomy (NHS guidelines (CHS132)). Participants were seated for 15 minutes prior to blood samples being taken, to allow plasma volume to settle. The area to be sampled was cleaned using an alcohol wipe and left to dry naturally. Samples were taken from the medial cubital vein of the non-dominant arm directly into vacutainers. Once the sample had been collected, the needle was disposed of and a lint free tissue was used to apply pressure to the area to restrict further bleeding. All blood samples were rested at room temperature for 75-90 minutes to allow clotting, and were then spun down in the centrifuge at 1,300 G for 11 minutes at 4°C and the serum was separated from the red blood cells. The serum was stored at -80°C, prior to analysis. All blood samples were analysed for creatinine, ALP, Osteocalcin and NTX.

3.5.2 Blood Analysis

After samples were collected, they were frozen at -80°C (as above). Once all samples had been collected, they were transported to the Department of Life Sciences, Swansea University, accompanied by the investigator. All blood analyses were performed on-site at Swansea University.

3.5.2.1 *Creatinine*

Serum creatinine was measured using the Randox RX Daytona Plus (Randox Laboratories Ltd., County Antrim, UK). Creatinine in alkaline solution reacts with picrate to form a coloured complex. Randox Daytona uses direct photometry to measure the rate of formation of the complex.

Before beginning the assay, reagents were brought to between 8°C and 15°C. Cuvettes containing samples were loaded into the auto-sampler unit. Reagents were loaded into the reagent container unit, where they were maintained at 8°C. Absorbance of the reaction liquid was measured every 13 seconds.

3.5.2.2 *Alkaline Phosphatase*

As with creatinine, serum alkaline phosphatase was measured using the Randox RX Daytona Plus (see 3.5.2.1).

3.5.2.3 *Osteocalcin*

Serum osteocalcin was measured using a Quantikine Enzyme linked Immunoabsorbent Assay (ELISA) kit for human osteocalcin (R&D Systems, Inc., Minneapolis, USA). Assays were performed using 96-well plates.

Prior to any assays being performed, all reagents and samples were brought to room temperature (8-25°C) and were prepared according to assay instructions. 100 µl of assay diluent was added to each well, followed by 50 µl of the sample serum. Wells were then covered, and were incubated for 2 hours at room temperature on a microplate shaker at 500rpm. After the incubation period, wells were aspirated and washed 4 times. Any remaining wash buffer was removed by inverting the plate and blotting against clean paper towels. 200 µl of human osteocalcin conjugate was added to each

well, and wells were covered, and incubated for 2 hours at room temperature on a microplate shaker. After the incubation period, wells were aspirated and washed 4 times. Any remaining wash buffer was removed by inverting the plate and blotting against clean paper towels. 200 µl of substrate solution was added to each well. Plates were covered to protect the wells from light, and were incubated for 30 minutes at room temperature on the benchtop.

After the incubation period, 50 µl of stop solution was added to each well. The optical density of each well was then determined within 30 minutes of the addition of stop solution, using a microplate reader (Crocodile Mini Workstation [Titertek-Berthold, Pforzheim, Germany]) with Crocodile Central Software (Titertek-Berthold, Pforzheim, Germany) set at 450nm.

3.5.2.4 *N-Teleopeptides*

Serum NTX was measured using an ELISA kit (Elabscience Biotechnology Co., Ltd., WuHan, China). Assays were performed using 96-well plates.

Prior to any assays being performed, all reagents and samples were brought to room temperature (8-25°C) according to assay instructions. 100 µl of sample serum was added to each well. Wells were covered, and incubated for 90 minutes at 37°C. After the incubation period, excess liquid was removed from the wells. 100 µl of Biotinylated Detection Ab working solution was added to each well, and wells were covered. Plates were incubated for 1 hour at 37°C. After the incubation period, wells were aspirated and washed 3 times. Any remaining wash buffer was removed by inverting the plate and blotting against clean paper towels. 100 µl of HRP conjugate was added to each well. Wells were covered, and incubated for 30 minutes at 37°C. After the incubation period, wells were aspirated and washed 5 times. Any remaining wash buffer was removed by inverting the plate and blotting against clean paper towels. 90 µl of substrate solution was added to each well. Plates were protected from light, and incubated for 15 minutes at 37°C.

After the incubation period, 50 µl of stop solution was added to each well. The optical density of each well was then determined immediately after the addition of stop solution, using a microplate reader with Crocodile Central Software set at 450nm.

Section 1:

Validation of DXA Measures of
BMD and Body Composition

4 Study 1: Reliability of DXA Whole Body, Hip, and Spine Scans

The first study conducted for this thesis establishes the reliability of the current institution's DXA scanner in order to quantify error for its use in subsequent studies measuring changes in body composition measures over time. This study sets out to achieve Objective 1, to quantify the error of the current institution's DXA scanner from repeated scans, as laid down in the Introduction and Literature Review (see 2.9.2).

4.1 Abstract

Introduction: *In order to track changes in body composition and BMD, change over time should be monitored rather than absolute BMD values (Alves et al., 2011). DXA is widely used for diagnosis and monitoring of disorders such as osteoporosis, and to measure body composition in a healthcare setting as well as to monitor effects of exercise training. In order to monitor changes in BMD or in body composition, DXA reliability must be established. DXA scans performed on the same scanner are reliable for assessing change in BMD over time in clinical population (Peel, 1995, Hangartner, 2007). The International Society for Clinical Densitometry (ISCD) recommends that precision calculations are performed on serial BMD measurements to establish the normal error of individual machines and operators. The present study therefore aims to quantify the error margin of the DXA scanner at the present institution, and to quantify the error range of DXA to measure changes in BMD and body composition for use in future longitudinal studies.*

Methods: *Fifty healthy adult women (40.7 ± 8.3 years; 73.1 ± 12.4 kg; 166.6 ± 4.4 cm) took part in the study. Participants underwent a DXA scan of the proximal femur, the lumbar spine, and the whole body. After the scans, participants stood up and walked around the room for five minutes, before being repositioned. Participants were then repositioned, and each scan was repeated. All data were analysed using SPSS for Windows. Root mean square (RMS) was calculated to measure variation between data sets, and least significant change (LSC) to establish the necessary change for future studies to be classed as significant. Alpha level was set at 0.05.*

Results: *Lumbar spine BMD; RMS SD was 0.01g.cm-2, LSC was 0.02g.cm-2, and %LSC was 1.96%. Proximal femur BMD; RMS SD was 0.01g.cm-2, LSC was 0.01g.cm-2, and %LCS was 1.50%. Femoral neck BMD; RMS SD was 0.02g.cm-2, LSC was 0.04g.cm-2, and %LSC was 4.94%. There was very strong positive correlation between mass measured by scales and by DXA ($r=0.99$, $p<0.001$). %LSC for subtotal body fat mass was 3.74%. %LSC for subtotal body lean mass was 2.33%. Calculated RMS SD and LSC were compared to guidelines set down by ISCD.*

Conclusion: *Calculated LSC and RMS were within ISCD guidelines for minimal acceptable precision for lumbar spine, proximal femur, and for femoral neck. This DXA was therefore sufficiently reliable to be used to monitor changes in BMD and body composition. In order to be attributable to actual changes, any changes observed in future studies should exceed the LSC.*

4.2 Introduction

Osteoporosis is an increasingly prevalent bone degenerative disease characterised by low bone mineral density (BMD). Bone is a dynamic, living tissue, which is constantly broken down and rebuilt throughout life, a process known as bone turnover. It is rebuilt and strengthened along lines of stress, meaning that it adapts according to daily lifestyle. Bone turnover is regulated by hormones. In women, the change in hormone level, in particular the drop in oestrogen, which occurs during and after the menopause, can lead to an imbalance in bone turnover. This means the breakdown of bone exceeds the building of bone, and so a loss in bone mass occurs. This loss of bone mass often leads to microarchitecture changes and an increase in fracture risk, and ultimately to osteoporosis.

In the UK, there are approximately 300,000 fragility fractures every year, with 1 in 2 women and 1 in 5 men over the age of 50 suffering a fracture at some point in their lives (National Osteoporosis Society, 2014). Hospital and post-fracture care costs the NHS approximately £2.3 billion per year for hip fractures alone. The World Health Organisation (WHO) define as osteoporosis as a t-score below -2.5, or more than 2.5 standard deviations below the mean peak bone mass (for young, healthy adults) as calculated by Dual Energy X-Ray Absorptiometry (DXA). Prior to the onset of osteoporosis, individuals develop low bone density, which is defined as having a BMD value greater than 1 standard deviation below the mean for the population. The International Society for Clinical Densitometry (ISCD) guidelines are to use z-scores rather than t-scores in premenopausal women and in men under the age of 50; a z-score of -2.0 or lower in these groups, in the absence of fragility fracture or other risk factors, should be described as “below the expected range for age”.

DXA is widely used in clinical settings for the diagnosis and monitoring of disease states such as osteoporosis. It is used to measure body composition and BMD in a healthcare setting, and more commonly to determine fracture risk as well as WHO classification for bone status. Since 2004, DXA has been included by the National Centre for Quality Assurance as a measure of the quality of osteoporosis management.

It has been suggested (Cawthon, 2013) that there is too much intra-individual variation in absolute BMD values to allow a single measure to be used for diagnosis. In addition

to this, Alves et al. (2011) put forward the idea that small measurement errors in DXA are unimportant, as long as the change over time is monitored rather than absolute values being used.

It can be seen then, that in order to monitor change in BMD over time, the reliability of DXA must first be established. It has been demonstrated (Pandya et al., 2008) that DXA is a highly reliable long-term measurement tool in patients suffering from Myotonic Dystrophy, and that values taken several months apart on the same individual can be directly compared to assess change in BMD. However, in order for these results to be directly comparable, it is necessary that the scans be performed on the same scanner (Peel, 1995). In support of this, Kohrt (1995) found that the values obtained from different manufacturers' scanners were not directly comparable to one another. The International Society for Clinical Densitometry (ISCD) recommends that precision calculations are performed on serial BMD measurements, and that a Least Significant Change (LSC) value is calculated in order to establish the normal error of the machine, and therefore establish what value changes in BMD measurements need to exceed in order to be considered significant.

Results have shown (Cummings, 2002, Tothill, 2007) that the short-term reliability of DXA is sufficient to monitor changes in BMD. However, it has also been suggested that the long-term reliability of DXA is much worse than the short-term reliability (Tothill, 2007). Patel (2000) on the other hand demonstrated that the long-term reliability was not significantly worse so as to prohibit the use of DXA as a long-term measure. In addition to this, Hangartner (2007) showed that the assumption of perfect long-term precision was appropriate for a Hologic scanner.

Within the current PhD, the DXA will be used to assess BMD values, and to determine changes in BMD and body composition measures during longitudinal studies. Therefore, following ISCD guidelines and to calculate LSC, it is necessary to establish the error of the DXA at the current institution. This will allow future studies to determine whether any change observed from scans could be due to the systematic or random error of the scanner, or whether changes represent statistical or clinical significance.

The purpose of the current study is therefore to investigate the test/retest reliability of this institution's Hologic Discovery A Scanner with QDR software, and the variability of the current investigator in analysing scans. This study aims to justify the use of DXA to measure changes in BMD and body composition measures in future longitudinal studies, and to quantify the error margin of the current institution's DXA scanner by calculating LSC.

4.3 Methods

4.3.1 Participants

Fifty Caucasian female participants aged between 30 and 57 (40.7 ± 8.3 years, 73.1 ± 12.4 kg, 166.6 ± 4.4 cm) were recruited. None had previously been diagnosed with bone degenerative diseases such as osteoporosis, or with low bone density. All participants were scanned within 10 days of their first day of menstruation. Perimenopausal women were defined as having irregular periods after years of a regular cycle; women in this category were scanned only during the months that menstruation did occur, and only during the first 10 days after the onset of menstruation. Postmenopausal women were defined as having no menstruation for 12 consecutive months, and could be scanned on any date. All participants were given full written and verbal information about what would be involved in taking part in the study, and written informed consent was obtained from each participant prior to any test being performed. Ethical approval was granted by Aberystwyth University. Participant information and informed consent forms are presented in Appendices B and C, respectively.

The average age and body fat percentage of participants (body fat $36.6 \pm 6.1\%$), were comparable to previous studies investigating the use of DXA for BMD and body composition. These values are also representative of the populations which will be used in future studies.

4.3.2 Protocol

Dual-Energy X-ray Absorptiometry scans were performed using a Hologic Discovery A QDR (APEX System Software Version 3.4.2). The participant completed a medical questionnaire (Appendix D. DXA radiation exposure questionnaire) to ensure that they met the Institutional ethical requirements for undergoing a DXA scan and that they

were within the annual radiation limits of the UK Government (Health and Safety Executive). They were then asked to wear only lightweight clothing, and to remove any items containing metal (e.g. underwire bra). Hospital scrubs were provided as alternative clothing if necessary. The participant then lay in the supine position on the scan table. A whole body scan, a lumbar spine scan, and a proximal femur scan were then performed, respectively. Once each scan had been completed, the participant was asked to stand up and was given five minutes to move around the room. When they were ready they were asked to lie on the bed again, were repositioned, and each scan was repeated. For more information on how scans were conducted, see 3.3.3.

Body mass was measured for each individual using scales (Seca 899, Seca gmb & co, Hamburg, Germany), so that the correlation of total body mass as measured by DXA and by scales could be established.

4.3.3 Scan Analysis

After all scans for all participants has been completed, they were analysed by the investigator within a 12-hour period. The automatic hip and spine scan analyses were adjusted to ensure that they were properly segmented. For the proximal femur scan, the region of interest (RoI) included the lesser trochanter, the greater trochanter and the insertion of the ball and socket joint. For the lumbar spine scan, the RoI included L1, L2, L3, and L4. The automatic segmentation lines were manually adjusted to ensure that they ran between each vertebra. Manual adjustments were made to the automatic analysis by adding or removing necessary bone pixels, or by adjusting the position of the neck box on the proximal femur scan.

The whole body scans were manually segmented into regions; head, trunk, left arm, right arm, left leg and right leg. For the purposes of analysis, the arms and legs were also combined into regions, “upper limbs” and “lower limbs”. This involved addition of the components “fat mass”, “lean mass” and “total mass”, and recalculation of the percentage fat. Statistical tests were then performed on these regions as on the other regions.

4.3.4 Statistical Analysis

All data were analysed using SPSS (SPSS for Windows, Version 21.0, SPSS Inc., Chicago). The two data sets were tested for normality, and paired sample t-tests, with an alpha level of 0.05, used to determine whether there were any significant differences between the results for the two scans. Limits of Agreement (LoA) were calculated to establish the variation of the results (Equation 1), along with standard error of the mean (SEM) to quantify the variability between the means of the pairs of data sets (Equation 2). Root mean square standard deviation (RMS SD) was calculated to measure the magnitude of the variation between the data sets (Equation 3), and least significant change (LSC) was calculated to establish what value future changes should exceed in order to be counted as statistically significant (Equation 4). Both RMS SD and LSC were calculated as set out in ISCD guidelines.

95% LoA were calculated as shown in Equation 5.

Equation 5: Formula used for calculation of 95% LoA

$$LoA = \bar{x} \pm 2s$$

Where \bar{x} is the mean of the sample, and s is the standard deviation of the sample.

SEM was calculated as shown in Equation 6.

Equation 6: Formula used for calculation of SEM

$$SE_x = \frac{s}{\sqrt{n}}$$

Where s is the standard deviation of the sample, and n is the number of observations in the sample.

RMS SD was calculated as shown in Equation 7.

Equation 7: Formula used for calculation of RMS

$$RMS\ SD = \sqrt{\frac{\sum(T2 - T1)^2}{n}}$$

Where $T1$ is the measurement taken in scan 1, $T2$ is the measurement taken in scan 2, and n is the number of pairs of scans in the sample.

LSC was calculated at the 95% confidence level as shown in Equation 8.

Equation 8: Formula used for calculation of LSC

$$LSC = RMS\ SD \times 2.77$$

In line with ISCD guidelines, RMS SD and LSC were calculated as absolute values. However, ISCD recommendations for acceptable precision error are given in terms of %CV and %LSC at the 95% confidence interval. %CV and %LSC are therefore presented in this study for the purposes of comparing results to the given guidelines.

%CV was calculated as shown in

Equation 9.

Equation 9: Formula used for calculation of %CV

$$\%CV = 100 \times \sqrt{\frac{\sum (s^2 / \bar{x})}{n}}$$

Where s^2 is the variance of the sample, \bar{x} is the mean of the sample, and n is the number of observations in the sample.

%LSC was calculated as shown in Equation 10.

Equation 10: Formula used for calculation of %LSC

$$\%LSC = \%CV \times 2.77$$

RMS SD, LSC, %CV, and %LSC calculated using the above equations were subsequently verified by calculating the same values using the ISCD precision calculator.

4.4 Results

The short-term variability of whole body, lumbar spine, and proximal femur measurements was calculated for repeated DXA scans. Fat mass, lean mass, total mass, and percent fat as calculated for scan 1 and scan 2 are presented in Table 4.1. T-tests indicated that there were no statistically significant differences in measures of fat mass or of lean mass between each whole body scan ($p > 0.05$). There was a statistically

significant difference between scan 1 and scan 2 for trunk total mass and for subtotal whole body (total body less head [TBLH]) total mass.

The %LSC for fat mass values obtained from the whole-body scan ranged from 3.74% to 11.15% (Table 4.1). %LSC for lean mass values obtained from the whole-body scan ranged from 2.33% to 9.96%. The left arm showed the largest %LSC for both fat mass and lean mass, and the whole body showed the smallest %LSC for both fat mass and lean mass.

BMC and BMD for scan 1 and scan 2 are presented in Table 4.2. T-tests indicated that there were no statistically significant differences in BMC or in BMD between scans ($p > 0.05$). %LSC for the proximal femur ranged between 1.50% and 4.63%, with BMC varying the most. %LSC for the femoral neck ranged between 4.94% and 11.89%, with BMC varying the most. %LSC for the lumbar spine ranged between 1.96% and 4.60%, with BMC varying the most. %LSC is presented as a % of the grand mean, although it is recommended that for future studies the absolute values, RMS SD and LSC, be used to establish significant changes

Table 4.1: Test 1 and Test 2 for whole body regional variables. * indicates significant differences between scan 1 and scan 2 at alpha level 0.05.

Region	Test 1 (mean±SD)	Test 2 (mean±SD)	RMS	%CV	%LSC
Left Arm					
Fat Mass (kg)	1.45±0.40	1.45±0.42	0.06	4.03	11.15
Lean Mass (kg)	2.07±0.29	2.07±0.30	0.07	3.60	9.96
Total Mass (kg)	3.51±0.54	3.52±0.57	0.11	2.81	7.79
Percent Fat (%)	40.67±6.59	40.60±6.83	1.19	2.92	8.08
Right Arm					
Fat Mass (kg)	1.54±0.45	1.53±0.44	0.06	3.32	9.21
Lean Mass (kg)	2.28±0.31	2.28±0.32	0.06	2.71	7.50
Total Mass (kg)	3.82±0.62	3.81±0.61	0.09	2.10	5.82
Percent Fat (%)	39.76±6.53	39.63±6.59	1.03	2.56	7.09
Trunk					
Fat Mass (kg)	11.57±4.83	11.68±4.86	0.31	2.69	7.44
Lean Mass (kg)	23.36±2.89	23.44±2.90	0.38	1.51	4.18
Total Mass (kg)	34.93±6.88	35.11±6.89 *	0.33	0.98	2.71
Percent Fat (%)	31.99±7.43	32.14±7.50	0.78	2.47	6.84
Left Leg					
Fat Mass (kg)	5.42±1.59	5.40±1.58	0.08	1.59	4.40
Lean Mass (kg)	7.45±1.04	7.43±1.05	0.10	1.28	3.55
Total Mass (kg)	12.88±2.38	12.83±2.35	0.15	1.18	3.26
Percent Fat (%)	41.49±5.46	41.47±5.49	0.38	0.95	2.64
Right Leg					
Fat Mass (kg)	5.64±1.69	5.61±1.68	0.11	2.07	5.74
Lean Mass (kg)	7.69±1.02	7.66±1.04	0.12	1.64	4.54
Total Mass (kg)	13.33±2.45	13.27±2.47	0.20	1.57	4.35
Percent Fat (%)	41.64±5.61	41.61±5.48	0.45	1.17	3.25
Subtotal (TBLH)					
Fat Mass (kg)	25.63±8.46	25.67±8.45	0.34	1.35	3.74
Lean Mass (kg)	42.83±5.17	42.88±5.24	0.38	0.84	2.33
Total Mass (kg)	68.47±12.16	68.55±12.20 *	0.17	0.48	0.68
Percent Fat (%)	36.67±6.10	36.68±6.09	0.49	1.35	3.78
Total					
Fat Mass (kg)	26.71±8.51	26.75±8.50	0.35	1.32	3.65
Lean Mass (kg)	46.29±5.33	46.30±5.41	0.38	0.77	2.14
Total Mass (kg)	73.00±12.37	73.06±12.41	0.19	0.54	0.71
Percent Fat (%)	35.88±5.81	35.92±5.81	0.46	1.27	3.61
Upper Limbs					
Fat Mass (kg)	2.99±0.84	2.98±0.85	0.08	2.45	6.78
Lean Mass (kg)	4.35±0.58	4.35±0.61	0.10	2.26	6.26
Total Mass (kg)	7.34±1.14	7.33±1.16	0.12	1.47	4.08
Percent Fat (%)	40.19±6.49	40.10±6.64	0.87	2.18	6.03
Lower Limbs					
Fat Mass (kg)	11.07±3.28	11.01±3.25	0.16	1.55	4.28
Lean Mass (kg)	15.14±2.04	15.09±2.06	0.18	1.23	3.40
Total Mass (kg)	26.21±4.81	26.10±4.80	0.30	1.18	3.27
Percent Fat (%)	41.57±5.52	41.54±5.47	0.33	0.84	2.33

Table 4.2: Test 1 and test 2 for bone variables. * indicates significant differences between scan 1 and scan 2 at alpha level 0.05.

Region	Test 1 (mean±SD)	Test 2 (mean±SD)	RMS	LSC	%CV	%LSC
Femur Neck						
BMC (g)	4.29±0.86	4.26±0.86	0.16	0.43	4.29	11.89
BMD (g·cm ⁻²)	0.84±0.13	0.84±0.12	0.02	0.04	1.78	4.94
Proximal Femur						
BMC (g)	33.85±5.37	33.77±5.33	0.56	1.55	1.67	4.63
BMD (g·cm ⁻²)	0.97±0.11	0.97±0.11	0.01	0.01	0.54	1.50
Lumbar Spine						
BMC (g)	63.00±12.25	63.14±12.10	1.05	2.92	1.66	4.60
BMD (g·cm ⁻²)	1.05±0.14	1.05±0.14	0.01	0.02	0.71	1.96
Subtotal Whole Body						
BMC (g)	1827.11±286.60	1832.06±287.40	21.16	58.61	1.13	3.14
BMD (g·cm ⁻²)	1.01±0.09	1.00±0.09	0.01	0.03	1.15	3.18
Total Whole Body						
BMC (g)	2434.80±359.86	2432.32±354.43	35.50	98.35	1.29	3.58
BMD (g·cm ⁻²)	1.19±0.10	1.18±0.10*	0.02	0.05	1.36	3.78

The calculated %CV for fat mass ranged from 1.32% to 4.03%, with the least variation found in the total body (1.32%), and the most variation seen in the left arm (see Table 4.2). %CV for lean mass ranged from 0.77% to 3.60%, with the least variation again found in the total body and the most variation found in the left arm. %CV for total mass ranged between 0.48% and 2.81%, with the least variation found in the subtotal and the most variation found in the left arm. %CV for percentage fat ranged from 0.93% to 2.92%, with the least variation found in the left leg and the most variation found in the left arm. %CV was lower for both fat mass and lean mass of the combined upper limbs than for individual arms. %CV was lower for both fat mass and lean mass of the

combined lower limbs than for individual legs. The largest %CV found for the combined upper limbs was 2.45%, which was found in the fat mass measures. The smallest variation was found in the total mass measures (1.47%). The largest %CV found for the lower limbs was 1.55%, which was found in the fat mass measures. The smallest variation was found in the percent fat measures (0.84%). Paired sample t-tests did not show significant difference between the data sets for any of the fat mass, lean mass, or percent fat measures ($p > 0.05$).

Pearson's correlation showed very strong positive correlation between mass as measured by DXA and by scales ($r = 0.99$, $p < 0.001$) (Figure 4.1).

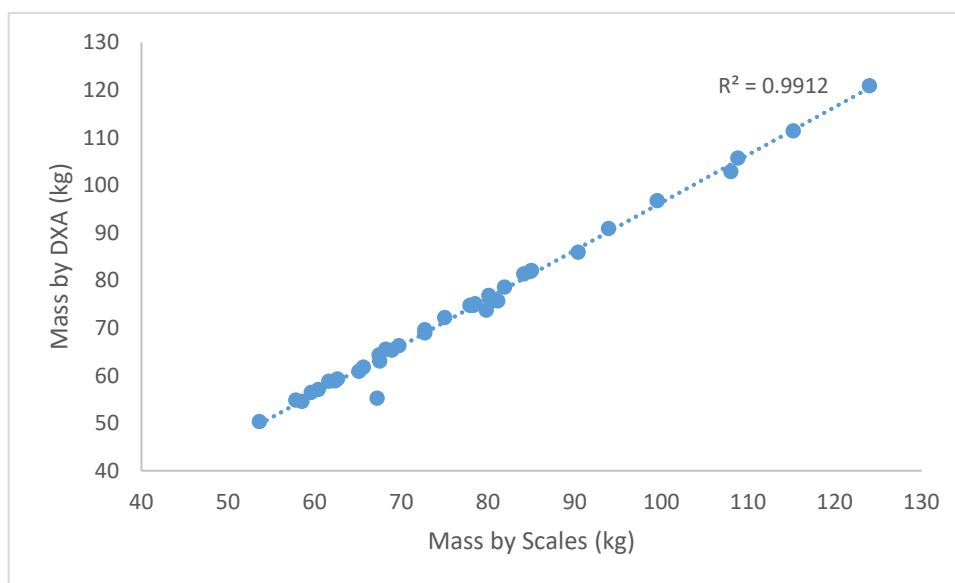


Figure 4.1: Correlation of mass measured by scales and by DXA ($r=0.96$)

4.5 Discussion

The current study aimed to examine the short-term reliability and LSC of the DXA whole body, proximal femur, and lumbar spine scans in the current laboratory by establishing the error of the machine, and LSC.

The International Society for Clinical Densitometry (ISCD) recommends that sufficient power is obtained for precision error calculations by scanning 15 patients 3 times, or scanning 30 patients twice. Root mean square (RMS) was calculated for each variable, as well as least significant change (LSC) for a 95% confidence interval. The LSC

shows the value which any difference in BMD must exceed in order to be counted as a statistical change, rather than due to methodological error. It is also recommended that, when used in future studies, precision error should be presented as absolute values, rather than as percentage changes (ISCD, 2015).

The ISCD states that the minimum acceptable precision for the lumbar spine by %CV is 1.9%, and LSC 5.3%. %LSC for the lumbar spine was calculated as 0.71%, and LSC was 1.96%, which is therefore within the acceptable precision range. Minimum acceptable range for the proximal femur is %CV stated as 1.8%, and LSC 5.0%. RMS for the total proximal femur was 0.54%, and LSC was 1.50%, which is therefore within the acceptable precision range. The minimum acceptable precision for the femoral neck is %CV stated as 2.5%, and LSC 6.9%. RMS for the femoral neck was 1.78%, and LSC 4.94%, which is therefore within the acceptable precision range. As the lumbar spine has the highest precision, and is also quick to respond to therapy, it is generally recommended that the spine scan is used in preference (ISCD, 2015). The proximal femur scan can also be used, although it is a less precise measurement, and the site is slower to respond to therapy than the lumbar spine.

DXA is a widely accepted method of measuring and monitoring BMD, as well as both fat mass and lean mass of soft tissue (Brownbill and Ilich, 2005, Kelly et al., 2009, Lee and Gallagher, 2008). This study aimed to investigate the short-term reliability of body composition; whether there was a significant difference between the obtained values for the fat mass, lean mass, or percentage fat for any of the anatomical segments. The results show a significant difference between the obtained values for trunk mass and subtotal mass ($p < 0.05$). Given that both the trunk and subtotal are bounded by the segment line across the neck, it is suggested that inaccuracies in the placement of the neck line may be causing these differences. Total whole body mass also showed lower RMS than subtotal values, demonstrating that the whole body composition values are more reliable than subtotal. This thesis will therefore use the total whole body mass rather than subtotal body mass to measure changes over time. There was no significant difference between the values for any other of the body composition variables ($p > 0.05$).

%CV for total fat mass was 1.32%. ISCD states that the minimum acceptable precision for total fat mass is 3%. The found precision is therefore within the acceptable range.

%CV for total lean mass was calculated as 0.77%. ISCD guidelines state that the minimum acceptable precision for total lean mass is 2%. The found precision is therefore within the acceptable range. ISCD guidelines state that the minimum acceptable precision for percent fat mass is 2%. %CV was calculated as 1.27%, which is therefore within the acceptable precision range.

Although the subtotal bone area of the whole body scans showed statistically significant differences between the two scans ($p < 0.05$), the BMD value did not show statistical difference ($p > 0.05$). The DXA can therefore be used to monitor changes in BMD. However, total whole body BMD showed significant differences between scan 1 and scan 2 (Table 4.2), demonstrating that the subtotal values are more reliable than total values; this supports the recommendation that the head be excluded from the whole body analysis, and subtotal be used instead (ISCD). The significant difference between the total body BMD values is not judged to be detrimental to the use of DXA to monitor changes in BMD, as future studies will use subtotal values rather than total values.

There were no significant differences between the results from the two scans with the exception of trunk area, trunk BMD, subtotal area, and total BMD, all of which are obtained from the whole body scan. Errors may occur during scanning of the trunk due to participant breathing during the scan, causing movement in the trunk area. The trunk will therefore not be used in future studies for measuring changes in BMD. The individual components of the trunk region (left rib, right rib, thoracic spine, and lumbar spine) demonstrate significant changes in right rib BMD and thoracic spine BMD only. Given that the lumbar spine BMD is analysed in a separate scan, individual trunk components will not be used for measuring changes in BMD over time. This error in measuring trunk BMD supports the use of separate scans for the lumbar spine.

The arms were grouped into the “upper limbs” region, and the legs were grouped into the “lower limbs” region. The upper limbs region showed a lower %CV for fat mass and lean mass than the individual segments; 2.26% and 2.45% for lean mass and fat mass, respectively, rather than ranging between 2.71% and 4.03% (right arm lean mass and left arm fat mass, respectively). The lower limbs region showed a lower %CV for each body composition measure than the individual leg segments; 1.23% and 1.55% for lean mass and fat mass, respectively, rather than ranging between 1.28% and 2.07%

(left leg lean mass and right leg fat mass, respectively). In support of this, Engelke (1995) demonstrated that precision of the DXA was increased by scanning larger areas. ISCD guidelines state that the minimum acceptable precision for measures of fat mass is 3%, and lean mass is 2% (ISCD, 2015). Individual right and left arms were not within these error limits, but combined “upper” limbs were within the acceptable precision range. In future studies therefore, grouped upper limb and lower limb regions will be used rather than the individual arms and legs.

Acceptable precision ranges are not recommended by ISCD for segmental body regions. Given that the whole body results are within the acceptable ranges, it is concluded that whole body scans can be used for monitoring changes in body composition over time. The calculated RMS and LSC for each region will be used in forthcoming studies to establish whether measured changes could be due to machine error or are true changes; any changes observed will need to exceed the LSC values in order to be significant changes.

Previous reports have stated that single measurements of BMD should not be used in diagnosis, and that multiple measurements tracking change across time are necessary (Alves et al., 2011, Cawthon, 2013). The findings of the present study demonstrate that the reliability of the current institution’s Hologic scanner is sufficient to allow repeat scans to be used to monitor changes in BMD over time. These findings are supported by previous work which has demonstrated that the short-term reliability of DXA is sufficient to allow monitoring of changes in BMD (Cummings, 2002, Tothill, 2007). Given previous reports that the assumption of perfect reliability is appropriate for a Hologic DXA scanner (Hangartner, 2007), these findings support the use of DXA in the diagnosis and monitoring of low bone density states.

Whole body mass as measured by DXA was shown to have very strong positive correlation with body mass as measured by scales, supporting the validity of DXA for use in measuring body mass. This is supported by previous findings that DXA is able to accurately calculate total mass in adults (Ackland et al., 2012, Toombs et al., 2012, Pineau et al., 2013). Economos et al (Economos et al., 1997) also demonstrated that, although DXA is able to accurately measure total mass, its accuracy in measuring individual soft tissue masses is variable. This agrees with the results of the current study, and provides support for establishing the machine and operator variability in

measurements for monitoring changes over time. In addition, this further supports the use of whole body values rather than subtotal body values to assess body mass.

4.5.1 Conclusions

The current study demonstrates that the DXA and the current operator have sufficient reliability to be used to monitor changes in body composition and BMD. There is sufficient reliability for the current institution's DXA to be used in forthcoming studies regarding changes in BMD and body composition over time. This study allowed calculation of the systematic and random error of the machine; any changes observed in future studies should exceed this error in order to be attributable to actual changes.

5 Study 2: The Effects of Creatine Supplementation on DXA Measurements of Body Composition and BMD

Following on from the previous study, the present study examines the validity of DXA during periods of supplementation with creatine monohydrate. This study was set out in order to qualify the use of DXA for measuring changes in body composition across periods of creatine supplementation. The present study sets out to achieve Objective 2, to investigate the effects of creatine supplementation on hydration status and DXA measure of BMD, fat-free mass, and fat mass (see 2.9.2).

5.1 Abstract

Introduction: *DXA is widely used for measuring and monitoring body composition in an athletic population. Creatine is increasingly used as an ergogenic aid (Tarnopolsky, 2010), and may result in increased water retention as it is taken up by the muscle (Candow and Chilibeck, 2008, Bemben et al., 2001). Alterations in hydration of fat-free mass may affect reliability of DXA scans (Nana, 2012, Nana et al., 2013) due to altered tissue density. The present study aims to investigate the effects of creatine supplementation on reliability of DXA measures of BMD and body composition. The study also aimed to establish whether creatine supplementation results in water retention, and whether retained water is attributed to fat mass or to fat-free mass.*

Methods: *Thirty-six adults (male=22, female=14; 20.5 ± 2.0 years; 77.0 ± 16.8 kg; 1.8 ± 0.1 m) took part in the study. Participants were randomly assigned to either a creatine supplement or a control. Participants randomised to the intervention group consumed $20\text{g}\cdot\text{day}^{-1}$ creatine monohydrate, while those in the control group consumed $20\text{g}\cdot\text{day}^{-1}$ of a sight- and taste-matched placebo for seven days. Body composition and BMD were measured by DXA at baseline, after supplementation, and after a one-week washout. Total body water was measured using BIA. Data were analysed using SPSS. T-tests were used to establish differences between groups at baseline values. Mixed between-within ANOVA was conducted to measure change in body composition, BMD, and body water volume across time between creatine and control groups. Alpha level was set at 0.05.*

Results: *Results showed no differences between baseline values (all $p > 0.05$). No significant changes in fat mass, fat-free mass, BMD, or body water were observed across time (all $p > 0.05$). There was no significant effect of supplement on measures of fat mass, fat-free mass, BMD, or body water (all $p > 0.05$). Significant positive correlations were observed between measures of change in mass by DXA and change in mass by scales ($r = 0.62$, $p < 0.001$); between change in mass by DXA and change in water volume ($r = 0.39$, $p = 0.024$); and between change in mass by scales and change in water volume ($r = 0.41$, $p = 0.018$).*

Conclusion: *Total mass by DXA is positively correlated with mass by scales. Creatine supplementation did not result in changes in body water volume. Creatine supplementation does not affect DXA measures of fat mass or fat-free mass over seven days' high-dose supplementation. DXA can be used to accurately monitor changes in body composition during short-term creatine supplementation.*

5.2 Introduction

DXA is widely used in athletic and clinical populations for measuring bone parameters, and for assessing body composition. DXA has been shown to be an accurate and reliable measure for measuring bone and soft tissue parameters, and also for monitoring changes in these parameters over time (Cummings, 2002, Hangartner, 2007, Pandya et al., 2008, Tothill, 1994). Several factors can cause artefacts in DXA scans, including the patient wearing metal or having any internal surgical pins, or the wearing of buttons. Clinical artefacts, such as aortic calcification, osteoarthritis, or fractures can also result in bone artefacts during DXA scans. Assessing changes in body composition over time is useful for monitoring disorders, as well as for measuring the effect of intervention programs. When monitoring changes over time, it is important that the reliability of the measure used is established in order to quantify whether changes observed could be attributed to precision errors of the measure.

Creatine is a naturally occurring nitrogenous compound which is obtained in the diet, and is used by the body for a variety of reasons, including in the anaerobic energy pathway. Supplementation with creatine is common practice in athletic populations because of its ergogenic effects (del Favero et al., 2012, Gualano et al., 2012, Smith et al., 2011, Tarnopolsky, 2010); when consumed alongside a resistance exercise program, it has been shown to result in increased muscle growth and improved performance when compared to an exercise program without supplementation (Candow et al., 2014b, Candow et al., 2015, Gualano et al., 2014a).

Creatine is taken up by the muscle via insulin-stimulated and sodium-dependent transporters, which creates active sodium and amino acid gradients across the plasma membrane, therefore drawing water into the cell, increasing cell hydration and osmolarity. Part of the increase in muscle mass seen during creatine supplementation may be attributable to water retention (Bemben et al., 2001, Candow and Chilibeck, 2007, Vukovich and Peeters, 2003, Ziegenfuss et al., 1998). Water retention occurs only during the early stages of supplementation; during continued supplementation, increases in dry mass occur following the intramuscular water retention (Hall and Trojian, 2013). The hydration levels of lean mass in the body are affected by intra- and extracellular water (St-Onge et al., 2004). Previous findings have demonstrated that

creatine supplementation appears to affect only intracellular water, while leaving levels of extracellular water unchanged (Bemben et al., 2001, Ziegenfuss et al., 1998).

DXA assumes a uniform relative hydration of fat free mass (FFM) at 0.73 units. Although the hydration status of fat free mass remains fairly constant in mammals (Wang et al., 1999), it is subject to variation. This variation of total body water in fat free mass will affect the validity of DXA measures. Large variation in muscle hydration status has been shown to affect the reliability of DXA scans (Horber et al., 1992, Pietrobelli et al., 1998); it is suggested that this is due to DXA attributing the increased water to either fat mass or to fat free mass. Given that water is of a lower density than FFM, increasing cell hydration of muscle tissue through creatine supplementation would result in decreased density of the tissue. In this way, DXA may measure some fat free mass as fat mass due to the changes in tissue density. Both fluid and food intake have been shown to be associated with increased error between repeat DXA measurement of soft tissue (Nana, 2012, Nana et al., 2013). High tissue hydration levels in children have also been shown to result in errors in calculation of fat percentage by DXA (Testolin et al., 2000), suggesting that the water is being attributed to FFM. In addition to this, Going et al. (Going, 1993) investigated the ability of DXA to detect small changes in soft tissue by inducing de- and rehydration. It was concluded that DXA was able to detect small changes in soft tissue in groups of participants, however it was not able to detect small changes within individuals. On the other hand, it has been demonstrated that errors in measurement of percentage fat by DXA were not due to changes in hydration of FFM (LaForgia et al., 2009, van der Ploeg, 2003).

The present study seeks to evaluate the use of DXA in forthcoming studies which investigate the effects of creatine supplementation on body composition measures. Therefore, the primary aim of the current study is to investigate the effects of creatine supplementation on the measures by DXA of both bone (BMC and BMD) and soft tissue composition (fat mass and fat free mass). The secondary aim is to establish whether any water retention caused by supplementation is attributed by DXA to lean mass.

5.3 Methods

5.3.1 Participants

Thirty-six Caucasian adult participants (20.5 ± 2.0 years; 77.0 ± 16.8 kg; 1.8 ± 0.1 m) (22 male, 14 female) took part in the study. Written informed consent was obtained from each participant prior to any test being performed. Ethical approval was granted by Aberystwyth University, in accordance with the Ethical Guidelines of the Helsinki Declaration of 1961 (revised in Fortaleza, 2013). Participant information sheets and informed consent forms are presented in Appendices E and F, respectively.

Inclusion criteria was individuals over the age of 18. Exclusion criteria were; those currently undergoing treatment for osteoporosis, those who had taken a creatine supplement in the 3 months previous, anyone who was or might be pregnant, anyone with renal function problems, and those with large metal implants (e.g. hip replacement).

5.3.2 Study Design

The study was a double-blind, placebo controlled trial. All participants attended the laboratory on three separate occasions, each one week apart. All measures were performed on each of these occasions. Seven days' of supplementation were taken following visit 1. After visit 2, participants had a one week "wash-out" period before their final visit.

Each visit for individual participants occurred at the same time each day. Participants were requested to refrain from eating or drinking for four hours before arriving at the laboratory, with a controlled fluid intake of 500ml water. Participants were asked to refrain from strenuous exercise or alcohol intake for the 24 hours prior to testing.

5.3.3 Protocol

5.3.3.1 *Anthropometric Measurements*

Participants' height was measured with a stadiometer without shoes and the minimum clothes to the nearest 0.1 cm (Harpndon Stadiometer [Holtain Ltd, Crymych, UK]). A scale with a 200 kg maximum capacity and 0.1 kg precision (Seca 899, Seca gmb & co,

Hamburg, Germany) was used to measure participants' mass, wearing lightweight clothing.

5.3.3.2 *Supplementation*

Participants were assigned in a randomised fashion, using block randomisation with a block size of 4, to the creatine or control group. Participants assigned to the creatine group (CRE) consumed $20\text{g}\cdot\text{day}^{-1}$ creatine monohydrate (Creapure, AlzChem, Trostberg, Germany), and those randomised to the control group (PLA) consumed $20\text{g}\cdot\text{day}^{-1}$ micro-crystalline cellulose (Blackburn Distributions, Lancashire, England). Participants consumed 10g of either creatine monohydrate or micro-crystalline cellulose twice daily with food, at the same times each day. Supplements were identical in colour and texture. Adherence was monitored by weighing of the supplement at the beginning and end of the study, and the amount of supplement consumed was calculated. One male participant was excluded from analysis because adherence to the study was below 80%.

5.3.3.3 *Nutrition Analysis*

Participants recorded a 3-day food diary of all food and drinks consumed for two weekdays and one weekend day prior to attending the lab; type, amount, and preparation of food was recorded. If this was not completed prior to attending the lab, they were asked to complete a retrospective 3-day food recall during their visit. Food diaries were analysed using WinDiets (WinDiets Professional 2008, The Robert Gordon University, Aberdeen) for macronutrients (fat, carbohydrate, and protein), and calcium intake. Creatine intake was calculated from food diaries, using the creatine content of the 10 highest creatine-containing foods.

Habitual creatine intake was calculated, and participants were split into groups based on median creatine intake; the half of the participants with lower creatine intake in the “lower” group, and the half with higher creatine intake in the “higher” group. There was a significant difference in creatine intake between the high and low intake groups ($p < 0.01$). There were no significant differences between any baseline body composition measures between those with a higher dietary creatine intake compared with a lower dietary creatine intake. There were no significant differences between values obtained at any time points (all $p > 0.05$).

5.3.3.4 *Physical Activity*

Participants completed the International Physical Activity Questionnaire (iPAQ) short version (www.ipaq.ki.se), to estimate habitual physical activity levels. The iPAQ is a standard and commonly used questionnaire, with acceptable validity for monitoring physical activity levels in healthy adults (Hagströmer et al., 2006).

5.3.3.5 *Dual energy X-ray absorptiometry*

At each visit, three DXA scans were conducted; the whole-body, hip, and spine. Bone area, bone mineral content, and bone mineral density were obtained DXA scan (Hologic Discovery QDR A; APEX System Software Version 3.4.2). The participant completed a medical questionnaire (Appendix D. DXA radiation exposure questionnaire) to ensure that they met the Institutional ethical requirements for undergoing a DXA scan and that they were within the annual radiation limits of the UK Government (Health and Safety Executive). Participants lay on the bed in the supine position, and proximal femur and L1-L4 spine positioning devices were used for the hip and spine scans, respectively. For the whole body scan, participants were asked to lie still in the supine position, and it was ensured that they lay within the scan area (for a more detailed procedure, see 3.3.3).

5.3.3.6 *Bioelectrical Impedance Analysis*

Total body water was obtained for each participant by Bioelectrical Impedance Analysis (BIA) (BodyStat 1500). Participants lay supine, with no parts of the body touching one another. Self-adhesive electrodes were applied to the right hand, behind the knuckle of the middle finger and on the wrist next to the ulnar head, and to the right foot, behind the 2nd toe and on the anterior ankle between the large bones protruding on either side of the joint.

5.3.4 *Analysis*

All data were analysed using SPSS (SPSS for Windows, Version 21.0, SPSS Inc., Chicago). Data were initially analysed for normality. T-tests were performed to establish differences between groups at baseline values. Mixed between-within ANOVA was performed in order to establish whether there were significant differences

in BMD and body composition measures between supplement and control groups across the period of supplementation. The within-subjects factor was time, and the between-subjects factor was supplement. Change across time was calculated as:

Post Intervention – Pre Intervention.

Pearson's r correlation was performed to establish correlation between change in body water (as measured by BIA), and change in mass by scales, and change in DXA measures of mass, fat mass, and fat-free mass. Data are presented as mean \pm standard deviation, with alpha level set at 0.05.

5.4 Results

There were no significant differences in height and weight between groups. Participant characteristics are summarised in Table 5.1.

The change across time was calculated (Table 5.2, Table 5.3). One-way ANOVA was conducted to compare these values between groups. No significant differences were found between any of subtotal fat mass, subtotal lean mass, subtotal mass, subtotal percent fat, mass by scales, percentage water (by BIA) and water volume (by BIA) between groups (all $p > 0.05$).

Table 5.1: Participant characteristics by group (PLA or CRE) and gender

	n		Height (m)		Weight (kg)		BMI (kg.m ⁻²)	
	PLA	CRE	PLA	CRE	PLA	CRE	PLA	CRE
Males	12	11	1.80±0.10	1.81±0.08	83.3±17.8	79.1±21.0	26.12±7.53	24.38±6.47
Females	8	7	1.66±0.06	1.66±0.04	69.9±12.9	71.1±6.8	25.23±4.07	25.63±1.72
Total	20	18	1.74±0.110	1.75±0.10	77.9±17.0	76.0±17.1	25.76±6.25	24.86±5.11

Table 5.2: Changes across time in measurement by DXA; difference between pre- and post-intervention values

	Fat Mass (kg)	Lean Mass (kg)	Subtotal Mass (kg)	Hip BMD (g·cm ⁻²)	Lumbar Spine BMD (g·cm ⁻²)
Female CRE	0.72±0.74	-0.53±0.99	0.19±1.21	-0.02±0.03	-0.01±0.02
Female PLA	0.27±0.50	0.10±1.14	0.37±1.37	0.00±0.02	0.01±0.04
Male CRE	-0.13±0.55	1.54±3.45	0.49±1.04	-0.01±0.02	0.00±0.06
Male PLA	0.08±0.50	-0.36±3.45	0.64±1.31	-0.03±0.14	0.01±0.05
CRE	0.15±0.50	-0.16±2.67	0.52±1.31	-0.02±0.03	0.01±0.06
PLA	0.20±0.74	0.74±2.90	0.37±1.08	-0.01±0.11	-0.00±0.04
Total	0.18±0.62	0.27±2.79	0.45±1.19	-0.02±0.08	0.00±0.05

Table 5.3: Changes across time in measurement by BIA; difference between pre- and post-intervention values

	Scales (kg)	Water Vol (kg)	Percent Water (%)
CRE	0.26±1.76	-0.23±1.31	-0.08±1.86
PLA	0.07±0.89	0.19±1.63	1.20±2.15
Female CRE	-0.11±0.82	0.03±1.35	0.13±1.28
Female PLA	-0.26±2.29	-0.26±1.02	0.91±1.91
Male CRE	0.18±0.95	0.25±1.78	0.15±2.45
Male PLA	0.64±1.22	-2.00±1.54	-0.81±1.53
Total	0.16±1.39	-0.04±1.46	0.02±1.97

Mixed between-within ANOVA revealed that there was no significant main effect of supplement on femoral neck BMD ($F(1,35)=1.95$; $p=0.17$; partial eta squared=0.053), proximal femur BMD ($F(1,35)=2.85$; $p=0.10$; partial eta squared=0.075), or lumbar spine BMD ($F(1,35)=3.74$; $p=0.06$; partial eta squared=0.096). There was no significant main effect of supplement on subtotal fat mass ($F(1,36)=0.62$; $p=0.44$; partial eta squared=0.017), subtotal fat-free mass ($F(1,36)=0.96$; $p=0.33$; partial eta squared=0.026), or subtotal mass ($F(1,36)=0.79$; $p=0.38$; partial eta squared=0.021). There was no significant main effect of supplement on water volume ($F(1,32)=0.09$; $p=0.76$; partial eta squared=0.003), or mass measured by scales ($F(1,35)=0.29$; $p=0.59$; partial eta squared=0.008).

Change in subtotal mass was positively correlated with change in subtotal fat mass ($r=0.40$, $p=0.014$), and with change in subtotal lean mass ($r=0.42$, $p=0.009$) (see Figure 5.1). Change in subtotal mass was correlated with change in mass by scales ($r=0.62$, $p<0.001$), and with change in water volume ($r=0.39$, $p=0.024$) (see Figure 5.2). Change in mass by scales was significantly correlated with change in water volume ($r=0.41$, $p=0.018$) (see Figure 5.3). No correlation was found between change in water volume and either change in subtotal fat mass or change in subtotal lean mass (both $p>0.05$).

Habitual creatine intake from food diaries was used as a covariate for mixed between-within ANOVA. There was no significant main effect of supplement on any measures

of BMD, fat mass, fat-free mass, total mass, water volume, or mass by scales when controlling for habitual creatine intake (all $p > 0.05$).

Mixed between-within ANOVA was conducted to assess the effect of supplement on BMD, body composition and body water between males and females. Within-subjects factor was time. Between-subjects factors were supplement and gender. There was no significant interaction of supplement and gender on subtotal fat-free mass ($F(3,33)=0.001$; $p=0.98$; partial eta squared=0.000), subtotal fat mass ($F(3,33)=0.18$; $p=0.67$; partial eta squared=0.005) or on subtotal mass ($F(3,33)=0.13$; $p=0.72$; partial eta = 0.004). There was no significant interaction of supplement and gender on BMD of the femoral neck ($F(3,33)=0.52$; $p=0.48$; partial eta squared=0.015), of the proximal femur ($F(3,33)=0.22$; $p=0.64$; partial eta=0.007), or of the lumbar spine ($F(3,33)=0.96$; $p=0.17$; partial eta=0.056). There was no significant interaction of supplement and gender on water volume ($F(3,30)=0.07$; $p=0.79$; partial eta=0.002) or on mass by scales ($F(3,33)=0.51$; $p=0.48$; partial = 0.015).

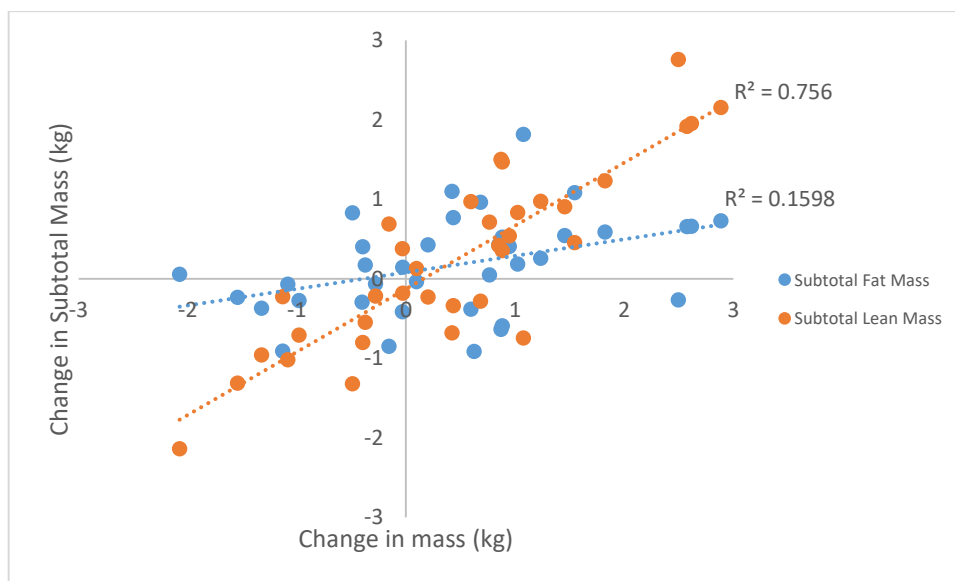


Figure 5.1: Correlation of change in subtotal mass with change in fat mass and change in lean mass

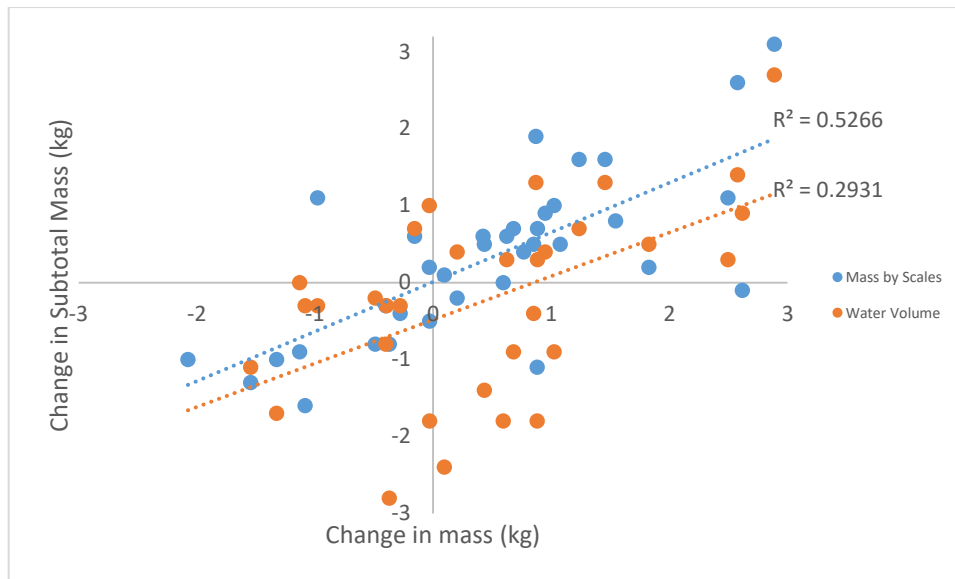


Figure 5.2: Correlation of change in subtotal mass with change in mass by scales and change in water volume

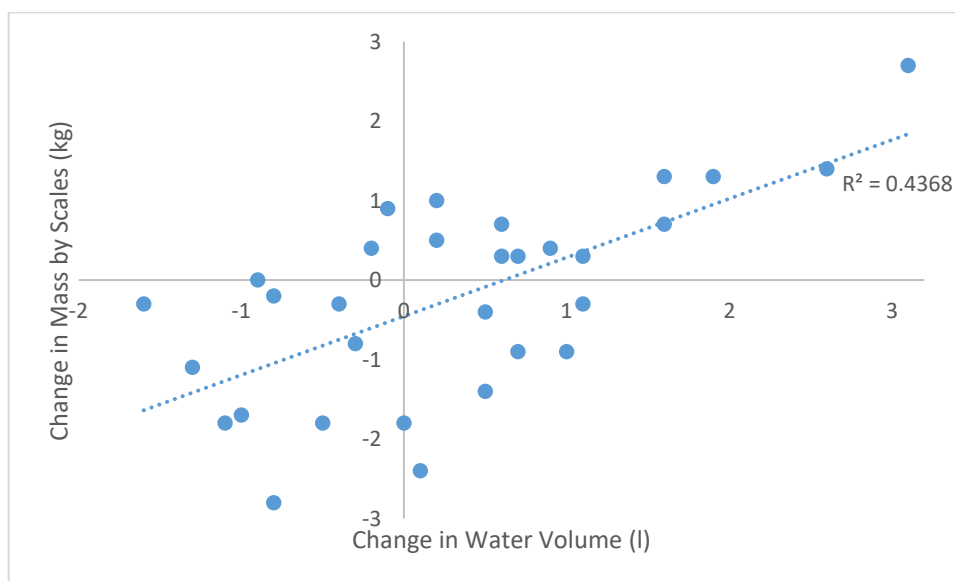


Figure 5.3: Correlation between mass by scales and change in water volume

5.5 Discussion

The current study aimed to investigate the effects of creatine supplementation on body composition measured by DXA, and to establish whether DXA assigned any additional water mass to either lean mass or fat mass. The present study also aimed to investigate

the effects of creatine supplementation on measures of BMD by DXA. To the author's knowledge, the effects of creatine supplementation on DXA measures of BMD or body composition have not previously been investigated. The present study was intended to establish whether reliable results for alterations in body composition would be obtained from this laboratory in future studies using oral creatine supplementation.

The findings of the present study demonstrate that there was no significant effect of creatine supplementation on DXA measures of fat free mass or fat mass, or on body water as measured by BIA. There was no effect of creatine supplementation on DXA measures of BMD. Difference in water volume was correlated with difference in subtotal mass by DXA, and with difference in scales. However, it was not correlated with either difference in subtotal fat mass or difference in subtotal lean mass. These results therefore suggest that slight changes in body water are detected by DXA, but that the increased fluid level is not attributed solely to either fat mass or to fat free mass, but is instead attributed partially to both.

To the author's knowledge, this is the first study investigating the direct effects of creatine monohydrate supplementation on DXA measures of BMD and body composition. Despite that, the current results are supported by findings that supplementation with creatine did not result in greater changes in body water than a placebo (Burke et al., 2003). Although the study by Burke et al (Burke et al., 2003) was carried out during an exercise program, there was no significant effect of creatine on body water.

Studies investigating the effects of creatine supplementation on body water in healthy adults have also demonstrated increases in body water following short-term supplementation (Lopez et al., 2009, Powers et al., 2003, Ziegenfuss et al., 1998). However, these studies routinely used athletic populations, who would have larger initial muscle mass and so an increased uptake and usage of creatine. This could therefore lead to larger effects of the supplementation in comparison to the present study, which investigated sedentary individuals.

In contrast to these findings, it has been demonstrated that increased levels of body water leads to errors in measurement of percent fat, fat mass, and fat free mass (Le Carvennec et al., 2007, Testolin et al., 2000, Vukovich and Peeters, 2003). However,

the methodology of these studies varied from that of the present study, by using children rather than adults (Testolin et al., 2000), or measuring body composition by ADP rather than by DXA (Le Carvennec et al., 2007, Vukovich and Peeters, 2003). Findings have also shown that fluid loss results in a decrease in measures of FFM and lean mass (Horber et al., 1992, Lukaski, 1997, Stenver et al., 1995), although these studies investigated patients undergoing dialysis, and consequently large fluid shifts, whereas the present study investigated healthy adults.

Previous studies finding that creatine supplementation resulted in increases in body water (Lopez et al., 2009, Powers et al., 2003, Ziegenfuss et al., 1998) used different methodologies from the present study, which could explain some of the differences found in the effects of hydration on DXA reliability. In addition, it is also possible that supplementation with creatine monohydrate, even at a high dose, does not result in as much water retention as was initially supposed. This lack of water retention would explain discrepancies between previous findings of the effects of water retention on body composition measures by DXA, and the present findings of no effects of creatine supplementation on DXA measures of fat mass or of fat free mass; actual changes in fat mass and fat free mass would not be expected over such a short duration.. In contrast to this supposition, creatine supplementation has been shown (Vukovich and Peeters, 2003) to lead to significant increases in water retention and subsequent increases in measurement of FFM by ADP; however, this study also used water consumption to increase body hydration levels, so it is likely that there were greater gains in body water than in the present study. It is also possible that the use of inactive participants in the present study in comparison to trained individuals resulted in a lower uptake of creatine into the muscle, and so a smaller effect of the supplementation.

The supplement given to participants was weighed at the beginning and the end of the week, and adherence to the dosage was measured. The amount of supplement consumed by participants over the course of the study was therefore established; all participants used in analysis had an adherence rate of 80% or greater. Factors such as diet and physical activity could affect the changes as a result of the supplementation, but were controlled as far as possible.

There were limitations to the present study. Despite being requested to refrain from exercise during the study, individuals who took part had differing habitual levels of

physical activity as measured by iPAQ. Those with higher normal levels of exercise would have higher muscle mass and increased muscle metabolism to begin with, and so would be likely to uptake the creatine more readily. Although participants were asked to provide a food diary, vegetarianism was not controlled for; vegetarians have lower dietary intake of creatine and so are likely to uptake more creatine at the onset of supplementation (Burke et al., 2003). Classification of vegetarianism based on food diaries could result in non-vegetarians being classified as vegetarian due to food diaries containing only 3 days' food recall. Additionally, the current study involved only 1 weeks' supplementation, although with a relatively high dose which averaged $0.25\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$. A longer period of supplementation could have resulted in natural changes in body composition being observed; significant changes were unlikely to occur naturally over only 1 week.

5.5.1 Conclusion

These findings demonstrate that DXA measures of body composition are unaffected by oral creatine supplementation. It is therefore concluded that DXA may be used as a method for monitoring body composition changes during a period of supplementation with creatine monohydrate. Despite anecdotal reports that creatine supplementation causes water retention, there was no evidence of water retention or of effects on measures of fat-free mass or fat mass by DXA.

Section 2:

Investigation of the Roles of
Creatine and Exercise in
Musculoskeletal Health

6 Study 3: The Relationships Between Habitual Creatine Intake, Physical Activity, BMD, and Muscular Strength in Adult Women

The third study undertaken in this thesis achieved Objective 3, to develop and understanding of the understanding relationships between measures of physical activity, creatine intake, bone density, fat-free mass, and functional muscle strength in adult women (2.9.2). This study set out to assess the relationships between creatine intake, physical activity, BMD, fat-free mass, and muscle strength.

6.1 Abstract

Introduction: *As muscles shorten, they create stress on the attached bone. Individuals who have greater muscle mass experience a greater pull of the muscle on the bone. According to Wolff's Law, this increased pull causes greater stress on the bones, resulting in fractures in the microarchitecture, and stimulating increased bone growth in the area. Supplementation with creatine results in increases in muscle mass (Candow et al., 2011), and when taken in conjunction with exercise promotes muscle hypertrophy beyond the effects of exercise alone (Volek, 1999). The present study aims to investigate the relationship between dietary creatine intake and BMD, and between dietary creatine and fat-free mass and muscle strength in adult women. The present study also aims to investigate the relationship between BMD, fat-free mass, and muscle strength.*

Methods: *One hundred and seven adult women (45.3 ± 13.2 years; $69.4 \text{ kg} \pm 12.8$ kg; 165.5 ± 6.2 cm; BMI $25.1 \pm 4.4 \text{ kg}\cdot\text{m}^{-2}$) took part in the study. BMD and body composition were measured by DXA. Isokinetic strength of the left leg at $60 \text{ deg}\cdot\text{sec}^{-1}$ and at $120 \text{ deg}\cdot\text{sec}^{-1}$ was measured by Biodex. Habitual creatine intake was calculated from three-day food diaries. Habitual physical activity was estimated from International Physical Activity Questionnaires. Data was analysed using SPSS for Windows. Correlation of BMD and leg strength were calculated. Physical activity levels and dietary creatine intake were used as covariates. Alpha level was set at 0.05.*

Results: *No significant correlation was found between dietary creatine intake and BMD of the femoral neck, of the proximal femur, or of the lumbar spine (all $p > 0.05$).*

No significant correlation was found between dietary creatine intake and lean mass of the lower limbs, the trunk, or subtotal body (all $p > 0.05$). No significant correlation was found between dietary creatine intake and leg strength (all $p > 0.05$). Significant positive correlation was found between lean mass and BMD of the femoral neck ($r = 0.37$, $p < 0.001$), of the proximal femur ($r = 0.31$, $p = 0.001$) and of the lumbar spine ($r = 0.43$, $p < 0.001$). Significant positive correlation was found between proximal femur BMD and leg strength at $60 \text{ deg}\cdot\text{sec}^{-1}$ ($r = 0.24$, $p = 0.013$) and $120 \text{ deg}\cdot\text{sec}^{-1}$ ($r = 0.25$, $p = 0.011$).

Conclusion: *There is no association between dietary creatine intake and BMD, fat-free mass, or muscular strength in adult women. Fat-free mass and muscle strength are positively associated with BMD.*

6.2 Introduction

Throughout life, bone is constantly being broken down and built up, a process known as bone turnover. Bone is broken down by osteoclasts, and built up by osteoblasts. When osteoblast activity exceeds osteoclast activity, bone growth occurs. Conversely, when osteoclast activity exceeds osteoblast activity, bone loss occurs. The state of osteoblast and osteoclast activity occurring at the same rate is known as bone homeostasis. The process of bone turnover is tightly controlled within the body. Wolff's Law states that bone growth is increased in areas of stress; as a bone undergoes more stress in day-to-day life, and micro fractures are formed, the amount of bone growth along lines of stress of that bone increases, leading to an increase in bone density at areas of high stress.

Individuals who have greater muscle mass experience a greater pull of the muscle on the bone. According to Wolff's Law, this increased pull causes greater stress on the bones, and so results in increased bone growth in the area. There would therefore be a resultant correlation between muscle mass and bone mineral density (BMD); in support of this theory, it has been demonstrated (Marwaha et al., 2014, Orsatti et al., 2011, Pasco et al., 2015, Snow-Harter et al., 1990) that muscle mass is positively correlated with BMD.

It has been found (Burt et al., 2012, Eser et al., 2009, Nichols et al., 1994) that individuals who undergo regular physical activity have a stronger correlation between muscle mass and BMD than sedentary individuals. Investigations into active individuals have shown that athletes with greater muscle strength have higher BMD (Helge and Kanstrup, 2002, Robinson et al., 1995). It has also been shown (Gruodyte et al., 2009) that vertical jump height is significantly correlated with BMD at the lumbar spine and the proximal femur in high-impact athletes. This effect of physical activity on the relationship between muscle and bone could also be explained by Wolff's Law; undergoing physical activity, in particular impact activity, causes the bones to undergo loading. In addition to this, the increased pull of muscle on bone during physical activity also results in increased loading to the bone, and so an increase in bone deposition in the region.

In contrast to the above findings, it has been demonstrated (Taaffe et al., 1997) that increases in muscle strength were not a factor in increases in lumbar spine and proximal femur BMD in college-aged females. It has also been shown (Eser et al., 2009) that muscle strength was not correlated with BMC in the lower limbs of retired elite athletes. Additionally, findings have demonstrated (Gruodyte et al., 2009) that there was no correlation between muscular strength and BMD of the lumbar spine and the proximal femur in moderate-intensity athletes, or in sedentary individuals. Previous findings have shown (Taaffe and Marcus, 2004) that there is a lower correlation between muscle mass and BMD in regular exercisers than there is in sedentary individuals. It is suggested that this could be explained by regular exercisers putting extra stress on the bones to the extent that the additional pull of extra muscle mass has only a negligible effect.

Creatine is a naturally occurring nitrogenous compound, which occurs in the diet; it is especially prevalent in red meat and fish. Creatine can also be synthesised in the body, although findings suggest that the synthesis of creatine results in lower levels of creatine being stored within the body than the intake of creatine from the diet (Bleiler and Schedl, 1962). Creatine is stored in the muscles as phosphocreatine (PCr), and is involved in the production of energy through the resynthesis of adenosine triphosphate (ATP).

It is possible to increase the body's stores of creatine through supplementation (Greenhaff et al., 1994). Increasing the body's PCr pool means that more energy can be expended before exhaustion sets in (Greenhaff et al., 1994), and that the body has a greater capacity to complete processes which have a high energy demand. Bone formation is a process which requires high amounts of energy (Gerber et al., 2005). Creatine supplementation in osteoblast-like cells increases the activation and mineralisation of the cells in vitro (Gerber et al., 2005). Supplementation with creatine also increases bone density in rats (Antolic et al., 2007) and in individuals suffering from Duchenne's muscular dystrophy (Tarnopolsky et al., 2004b).

Supplementation with creatine results in increases in muscle mass (Candow et al., 2011), and when taken in conjunction with exercise promotes muscle hypertrophy beyond the effects of exercise alone (Volek, 1999). This muscle hypertrophy may be due in part to water retention within the muscle; there is increased osmolality of the

muscle following supplementation (Alfieri et al., 2006). However, in longer term training programs water retention appears to be less likely to be a factor in muscle hypertrophy (Vandenberghe et al., 1997); in these case, muscle growth may be due to the larger creatine stores allowing more work to be performed and so promoting training adaptations (Francaux and Poortmans, 1999). Previous findings in this thesis have demonstrated no water retention following short-term, high-dose creatine supplementation, suggesting that any body composition changes observed following supplementation regimes are likely to be due to increased muscle growth (Chapter 5: Study 2: The Effects of Creatine Supplementation on DXA Measurements of Body Composition and BMD).

The primary aim of the present study is to investigate the relationship between habitual dietary creatine intake and BMD in adult women. The secondary aim of the present study is to investigate the relationship between dietary creatine intake and muscle mass, and creatine intake and muscular strength in adult women. The present study also aims to investigate the relationship between muscle mass and strength, and habitual levels of physical activity, and BMD. It was hypothesised that creatine intake would be positively correlated with both BMD and muscle strength, and that muscle strength would be positively correlated with BMD. It was further hypothesised that there would be a positive correlation between physical activity levels and muscle strength, and between physical activity levels and BMD.

6.3 Method

6.3.1 Participants

One hundred and seven adult women (45.26 ± 13.19 years; $69.37 \text{ kg} \pm 12.78 \text{ kg}$; 165.54 ± 6.23 ; BMI $25.31 \pm 4.43 \text{ kg} \cdot \text{m}^{-2}$) took part in the study (29 postmenopausal, 5 perimenopausal, 73 premenopausal). Before agreeing to take part in the study, all prospective participants were given an information sheet to read through, and the study was also explained verbally. Participants had the opportunity to ask any questions they may have about the study or about their involvement. Written informed consent was then obtained from each participant. Ethical approval was granted by Aberystwyth University, in accordance with the Ethical Guidelines of the Helsinki Declaration of 1961 (revised in Fortaleza, 2013). Participant information sheets and informed consent forms are presented in Appendices G and H, respectively.

Inclusion criteria was females over the age of 30. The exclusion criteria were; those currently undergoing medical treatment for osteoporosis, those who were or might be pregnant, those who had taken a creatine supplement in the 3 months previous, and those with large metal implants (e.g. hip replacement).

6.3.2 Study Design

The study was a cohort study. Participants attended only one visit to the lab, where all measures were taken and all questionnaires were completed.

6.3.3 Protocol

6.3.3.1 *Anthropometric measurements*

Participants' height was measured with a stadiometer without shoes and the minimum clothes to the nearest 0.1 cm (Harpender Stadiometer [Holtain Ltd, Crymych, UK]). A scale accurate to 0.1 kg (Seca 899, Seca gmb & co, Hamburg, Germany) was used to measure participants' mass.

6.3.3.2 *Nutrition Analysis*

Participants recorded a 3-day food diary of all food and drinks consumed; type, amount, and preparation. If this was not completed prior to attending the lab, they were asked to complete a retrospective 3-day food recall during their visit. Food diaries were analysed using WinDiets (WinDiets Professional 2008, The Robert Gordon University, Aberdeen) for macronutrients (fat, carbohydrate, and protein), and for calcium intake. Creatine intake was calculated from food diaries, using the creatine content of the 10 highest creatine-containing foods.

6.3.3.3 *Physical Activity*

Participants completed the International Physical Activity Questionnaire (iPAQ) short version (www.ipaq.ki.se), to estimate habitual physical activity levels. The iPAQ is a standard and commonly used questionnaire, with acceptable validity for monitoring physical activity levels in healthy adults (Hagströmer et al., 2006) (see Appendix G. iPAQ (short)).

6.3.3.4 *Dual energy X-ray absorptiometry*

Participants lay on the bed in the supine position, and hip and spine positioning devices were used for the BMD scans. For the whole body scan, participants were asked to lie still in the supine position. Bone area, bone mineral content, and bone mineral density were obtained for each participant by DXA scan (Hologic Discovery QDR A; APEX System Software Version 3.4.2) in supine position, of the proximal femur, lumbar spine and whole body (for a more detailed procedure, see 3.3.3).

6.3.3.5 *Isokinetic dynamometry*

Maximal strength production was assessed using an isokinetic dynamometer (Biodex Isokinetic System III, IPRS Mediquipe, Little Blakenham, UK). Participants were seated on the Biodex and securely strapped in. The left leg was held at a 90° angle, and participants' natural range of movement of the knee was established. They were then asked to complete 5 maximal voluntary concentric contractions of the quadriceps and the hamstrings of the left leg; this was repeated at 60deg·sec⁻¹ and 120deg·sec⁻¹ (for a more detailed procedure, see 3.4).

6.3.4 **Analysis**

All data were analysed using SPSS for Windows (SPSS for Windows, Version 21.0, SPSS Inc., Chicago). Data were initially tested for normality. Pearson's correlation was performed to establish the relationship between proximal femur BMD and leg strength as measured by DXA. Partial correlation was performed to establish the relationship between hip BMD and leg strength while controlling for habitual physical activity levels, and for dietary intake of calcium and creatine.

6.4 **Results**

Participant characteristics are presented in Table 6.1. No significant correlation ($p > 0.05$) was demonstrated between dietary creatine intake and BMD of the lumbar spine, the total hip, or the neck of the femur. No significant correlation was found between dietary creatine intake and lean mass of the lower limbs, the trunk, or subtotal body (all $p > 0.05$). No significant correlation was found between creatine intake and maximal

torque at $60\text{deg}\cdot\text{sec}^{-1}$ or at $120\text{deg}\cdot\text{sec}^{-1}$ (all $p>0.05$) of either the quadriceps or the hamstrings during concentric contraction.

When participants were split into groups based on menopausal status, significant positive correlation was found between creatine intake and femur neck BMD ($r=0.90$, $p=0.037$) and lumbar spine BMD ($r=0.90$, $p=0.037$), only in perimenopausal women ($n=5$).

Significant differences were found between menopausal status groups in lean mass and in maximal torque; postmenopausal women demonstrated significantly lower lean mass than premenopausal women in the upper limbs ($p=0.035$) and the lower limbs ($p=0.036$). Postmenopausal women showed significantly lower torque at $60\text{deg}\cdot\text{sec}^{-1}$ than premenopausal ($p < 0.001$) and perimenopausal women ($p=0.002$). Postmenopausal women showed significantly lower torque at $120\text{deg}\cdot\text{sec}^{-1}$ than premenopausal ($p < 0.001$) and perimenopausal women ($p=0.007$). No significant differences were found between peri- and premenopausal women in maximal torque ($p > 0.05$). No significant differences were found between menopausal groups in any other measure (all $p > 0.05$).

Table 6.1: Participants characteristics split by menstrual status. Post = postmenopausal, peri = perimenopausal, pre = premenopausal. * indicates significant differences between postmenopausal and perimenopausal women. ‡ indicates significant differences between postmenopausal and premenopausal women.

	Age (years)	Height (m)	Mass (kg)	BMI (kg·m ⁻²)	Creatine intake (g·day ⁻¹)	Femur Neck BMD (g·cm ⁻²)	Hip BMD (g·cm ⁻²)	Spine BMD (g·cm ⁻²)	Torque at 60deg·sec ⁻¹ (Nm)	Torque at 120deg·sec ⁻¹ (Nm)
Post	60.1±13.3	1.64±0.06	68.83±13.11	25.53±3.77	1.30±0.74	0.77±0.10	0.90±0.11	0.97±0.12	88.67±29.33 * ‡	70.46±21.43 * ‡
Peri	49.8±7.0	1.67±0.05	69.98±10.30	25.08±3.36	1.74±1.43	0.77±0.12	0.96±0.07	0.93±0.13	133.66±32.15 *	100.70±14.54 *
Pre	38.7±7.2	1.66±0.06	69.91±13.13	25.34±4.87	1.27±0.83	0.82±0.12	0.95±0.13	1.01±.11	119.89±25.19 ‡	90.41±19.79 ‡
Total	45.3±13.2	1.66±0.06	69.37±12.78	25.31±4.43	1.29±0.83	0.80±0.12	0.94±0.12	1.00±0.12	111.48±30.52	85.28±22.29

Significant correlation was demonstrated between BMD of the neck of the femur and; upper limb lean mass ($r=0.30$, $p=0.002$), lower limb lean mass ($r=0.33$, $p<0.001$), trunk lean mass ($r=0.38$, $p<0.001$), and subtotal lean mass ($r=0.37$, $p<0.001$) (see Figure 6.1).

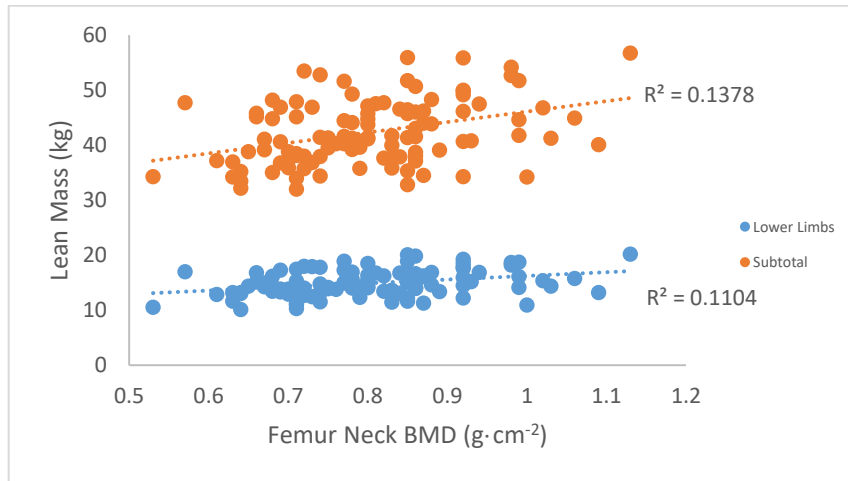


Figure 6.1: Femur neck BMD v. lower limb lean mass ($r=0.33$) and subtotal lean mass ($r=0.37$)

Significant correlation was demonstrated between total hip BMD and; upper limb lean mass ($r=0.31$, $p=0.001$), lower limb lean mass ($r=0.33$, $p=0.001$), trunk lean mass ($r=0.39$, $p<0.001$), and subtotal lean mass ($r=0.38$, $p<0.001$) (see Figure 6.2).

Significant correlation was demonstrated between BMD of the lumbar spine and; upper limb lean mass ($r=0.40$, $p<0.001$), lower limb lean mass ($r=0.40$, $p<0.001$), trunk lean mass ($r=0.42$, $p<0.001$), and subtotal lean mass ($r=0.43$, $p<0.001$) (see Figure 6.3).

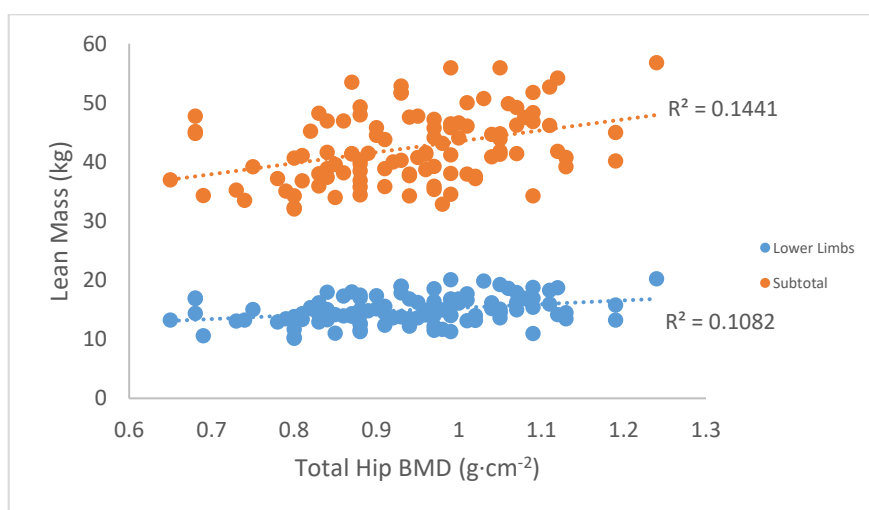


Figure 6.2: Total hip BMD v. lower limb lean mass ($r=0.33$) and subtotal lean mass($r=0.38$)

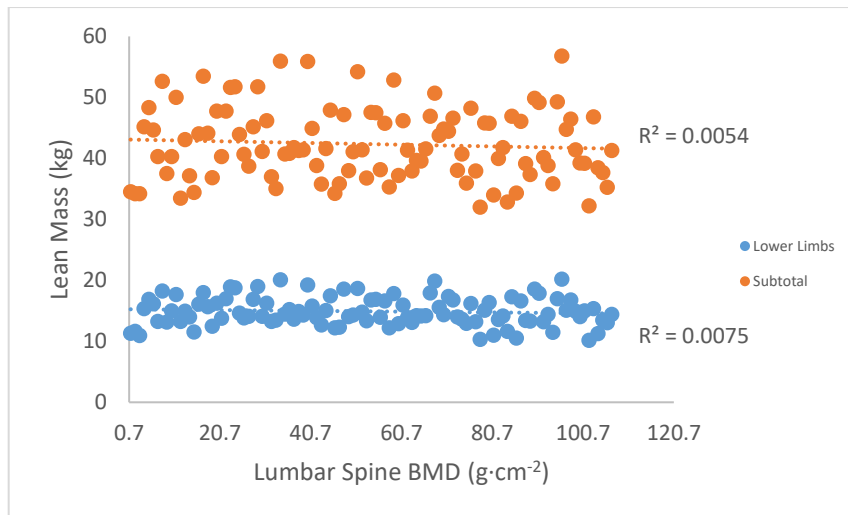


Figure 6.3: Lumbar spine BMD v. lower limb lean mass ($r=0.40$) and subtotal lean mass ($r=0.43$)

6.4.1 Premenopausal

Analysis by menopausal status demonstrated that in premenopausal women, significant correlations were found between femur neck BMD and lean mass of the upper limbs, the trunk, lower limbs, and subtotal whole body ($r=0.26$, $p=0.034$; $r=0.32$, $p=0.008$; $r=0.26$, $p=0.030$; $r=0.31$, $p=0.011$, respectively). Significant correlation was found between total hip BMD and lean mass of the upper limbs, the trunk, lower limbs, and subtotal whole body ($r=0.27$, $p=0.026$; $r=0.32$, $p=0.008$; $r=0.25$, $p=0.035$; $r=0.30$, $p=0.012$, respectively), and between lumbar spine BMD and lean mass of the upper limbs, the trunk, lower limbs, and subtotal whole body ($r=0.42$, 0.41 , 0.42 , 0.44 , respectively; all $p<0.001$).

6.4.2 Postmenopausal

In postmenopausal women, significant correlation was found between femur neck BMD and lean mass of the trunk, lower limbs, and subtotal whole body ($r=0.35$, $p=0.025$; $r=0.38$, $p=0.047$; $r=0.42$, $p=0.028$, respectively), but not with lean mass of the upper limbs. Significant correlation was found between total hip BMD and lean mass of the trunk, and subtotal whole body ($r=0.44$, $p=0.018$; $r=0.42$, $p=0.028$, respectively), but not with lean mass of the upper or lower limbs. Significant correlation was found between lumbar spine BMD and lean mass of the upper limbs, the trunk, and subtotal

whole body ($r=0.38$, $p=0.042$; $r=0.43$, $p=0.019$; $r=0.41$, $p=0.028$, respectively), but not with lean mass of the lower limbs.

Significant correlation was found between total hip BMD and maximal torque at $60\text{deg}\cdot\text{sec}^{-1}$ ($r=0.24$, $p=0.013$) and $120\text{deg}\cdot\text{sec}^{-1}$ ($r=0.25$, $p=0.011$) (see Figure 6.4), and between BMD of the neck of the femur and maximal torque at $60\text{deg}\cdot\text{sec}^{-1}$ ($r=0.25$, $p=0.010$) and $120\text{deg}\cdot\text{sec}^{-1}$ ($r=0.29$, $p=0.003$) (see Figure 6.5).

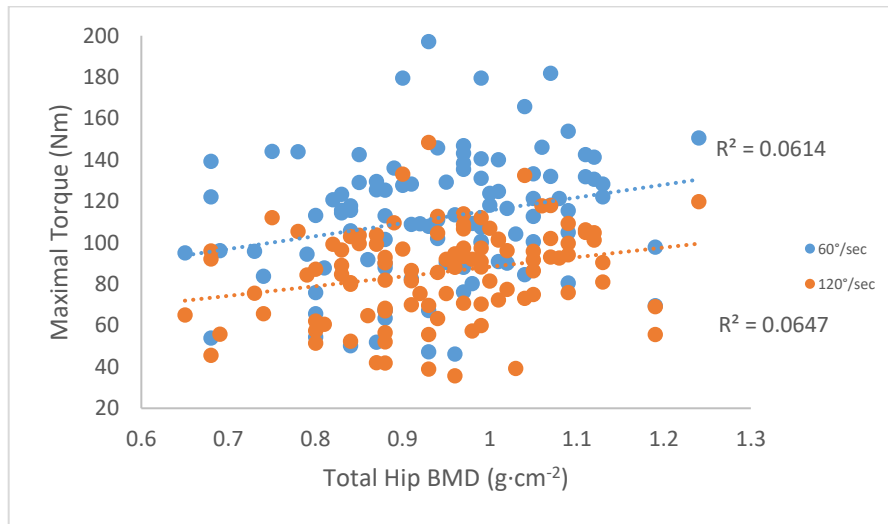


Figure 6.4: Total hip BMD v. maximal torque at $60\text{deg}\cdot\text{sec}^{-1}$ ($r=0.243$) and at $120\text{deg}\cdot\text{sec}^{-1}$ ($r=0.250$)

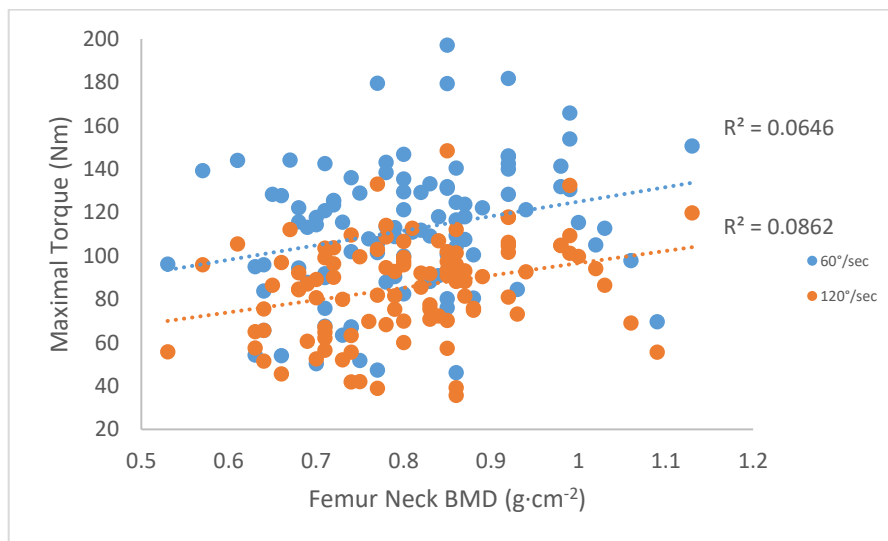


Figure 6.5: Femur neck BMD v. maximal torque at $60\text{deg}\cdot\text{sec}^{-1}$ ($r=0.251$) and at $120\text{deg}\cdot\text{sec}^{-1}$ ($r=0.291$)

When controlling for creatine intake, significant correlation was found between total hip BMD and maximal torque at 60 deg·sec⁻¹ ($r=0.25$, $p=0.012$) and 120 deg·sec⁻¹ ($r=0.26$, $p=0.008$), and between BMD of the neck of the femur and maximal torque at 60 deg·sec⁻¹ ($r=0.25$, $p=0.010$) and 120 deg·sec⁻¹ ($r=0.30$, $p=0.002$).

The iPAQ differentiates between vigorous physical activity, moderate physical activity, and walking. The iPAQ gives definitions of vigorous and moderate physical activity; the definition of vigorous physical activity is given as exercise which require hard effort and make the individual breathe much harder than normal, such as aerobics. The definition of moderate physical activity given as activities requiring moderate effort and making individuals breathe somewhat harder than normal, such as playing doubles tennis. No significant correlation was found between habitual physical activity as measured by iPAQ and maximal torque at 60 deg·sec⁻¹ or at 120 deg·sec⁻¹ (all $p>0.05$).

Significant correlation was demonstrated between lean mass and maximal torque at 60deg·sec⁻¹. Maximal torque at 60 deg·sec⁻¹ was significantly correlated with; upper limb lean mass ($r=0.32$, $p=0.001$), lower limb lean mass ($r=0.41$, $p<0.001$), trunk lean mass ($r=0.33$, $p=0.001$), and subtotal lean mass ($r=0.38$, $p<0.001$) (see Figure 6.6).

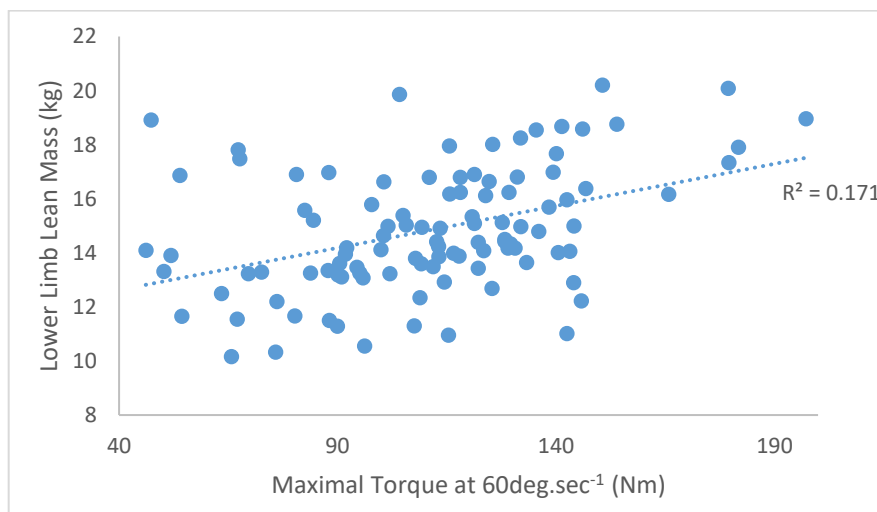


Figure 6.6: Torque at 60 deg·sec⁻¹ v. lower limb lean mass ($r=0.41$) for all participants

Significant correlation was demonstrated between lean mass and maximal torque at 120deg·sec⁻¹. Maximal torque at 120 deg·sec⁻¹ was significantly correlated with; upper limb lean mass ($r=0.27$, $p=0.006$), lower limb lean mass ($r=0.35$, $p<0.001$), trunk lean mass ($r=0.29$, $p=0.003$), and subtotal lean mass ($r=0.33$, $p=0.001$) (see Figure 6.7).

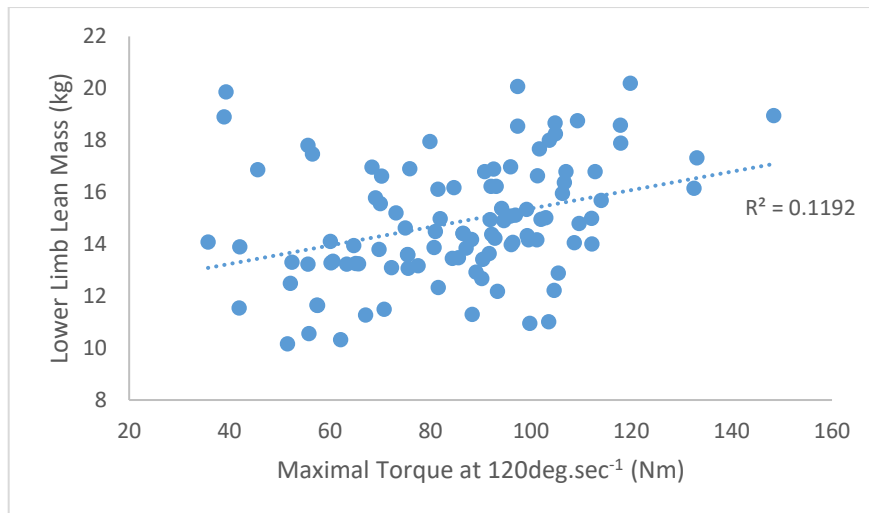


Figure 6.7: Maximal torque at 120 deg·sec⁻¹ v. lean mass of the lower limbs ($r=0.35$) for all participants

In premenopausal women, significant correlation was found between maximal torque at 60 deg·sec⁻¹ and lean mass of the trunk, lower limbs, and subtotal whole body ($r=0.30$, $p=0.014$; $r=0.37$, $p=0.002$; $r=0.34$, $p=0.006$, respectively) (see Figure 6.8), and between maximal torque at 120 deg·sec⁻¹ and lean mass of the trunk, lower limbs, and subtotal whole body ($r=0.26$, $p=0.038$; $r=0.30$, $p=0.016$; $r=0.28$, $p=0.023$, respectively) (see Figure 6.9). In postmenopausal women, no significant correlation was found between measures of lean mass and maximal torque at either 60 deg·sec⁻¹ or 120 deg·sec⁻¹ (all $p>0.05$) (see Figure 6.8, Figure 6.9).

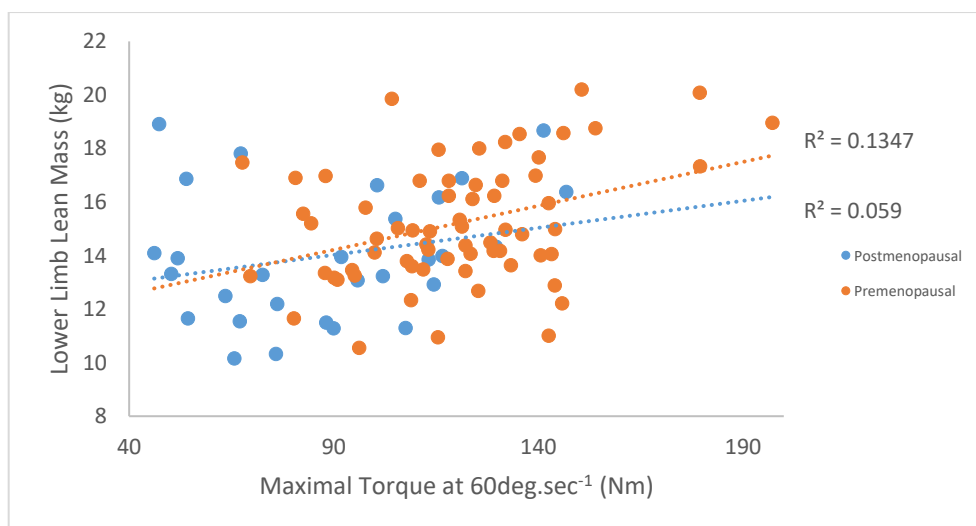


Figure 6.8: Torque at 60 deg·sec⁻¹ v. lower limb lean mass for postmenopausal ($r=0.24$) and premenopausal participants ($r=0.37$)

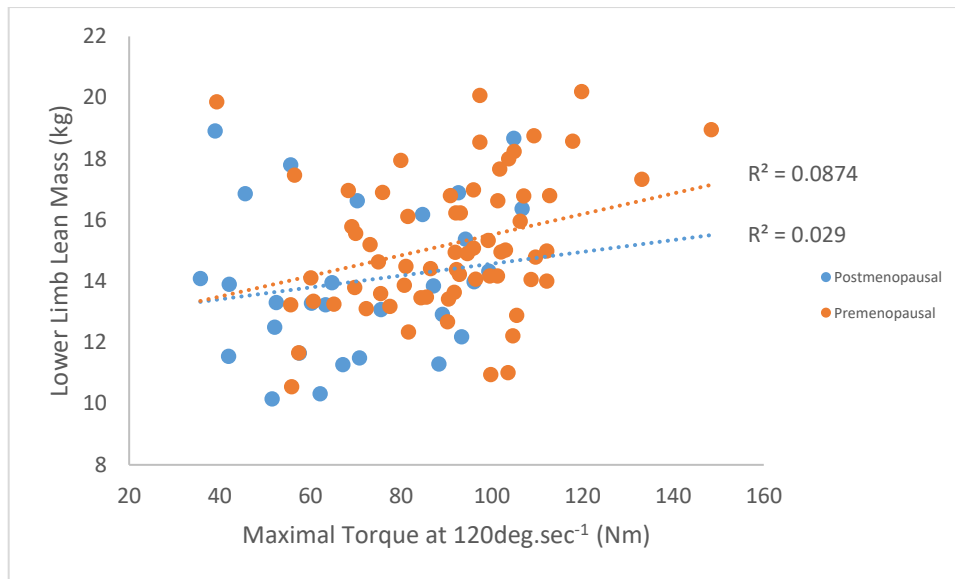


Figure 6.9: Maximal torque at 120 deg.sec⁻¹ v. lean mass of the lower limbs for postmenopausal ($r=0.17$) and premenopausal participants ($r=0.30$)

6.5 Discussion

The present study aimed to investigate the relationship between habitual creatine intake, BMD, and muscular strength. It was hypothesised that there would be a positive correlation between creatine intake and BMD, and between creatine intake and muscular strength. It was also hypothesised that there would be a positive correlation between BMD and muscular strength.

Present findings did not demonstrate that there was significant correlation between dietary creatine intake and BMD. This was supported by previous findings that there is no relationship of creatine with bone mass in postmenopausal women (Gualano et al., 2014a), or with bone health in rats used as experimental models for osteoporosis (Alves et al., 2012, Murai et al., 2015). BMD in individuals suffering from some forms of muscular dystrophy have also been shown to be unaffected by creatine supplementation (Tarnopolsky et al., 2004a).

In comparison, previous findings have shown that creatine supplementation improves measures of bone strength in postmenopausal women (Chilibeck et al., 2014), and results in a decrease in markers of bone resorption (Candow et al., 2008, Cornish et al., 2009), suggesting a change in the balance of bone turnover. Supplementation with

creatine results in increased bone density in individuals suffering from some types of muscular dystrophy (Tarnopolsky et al., 2004b) and in growing rats (Antolic et al., 2007).

However, these studies increased participants' normal ingestion of creatine; it may be that normal levels of dietary creatine are insufficient to significantly influence bone turnover. This would mean that additional creatine, in excess of dietary creatine, could be necessary to influence bone turnover. The aforementioned studies which used human participants included an exercise intervention (Candow et al., 2008, Chilibeck et al., 2014, Cornish et al., 2009) or else demonstrated the importance of physical activity in creatine uptake by the body (Tarnopolsky et al., 2004b). It is possible that participants in the current study were not active enough to sufficiently utilise their dietary creatine.

Present findings did not demonstrate that there was significant correlation between dietary creatine intake and muscle mass. These findings are supported by previous work which has demonstrated that creatine supplementation does not result in changes in fat free mass or lean mass in active women (Ferguson and Syrotuik, 2006, Kresta et al., 2014, Larson-Meyer et al., 2000). Individuals suffering from myotonic dystrophy type I also do not exhibit changes in lean mass following creatine supplementation (Tarnopolsky et al., 2004a).

In comparison, previous findings have shown that there is a positive correlation between creatine intake and muscle mass. Untrained individuals and older adults undergoing creatine supplementation above habitual dietary intake exhibit increases in lean mass (Brose et al., 2003, van Loon et al., 2003). Trained athletes also showed increasing lean mass following creatine supplementation (Becque et al., 2000, Earnest et al., 1995, Kirksey et al., 1999, Lehmkuhl et al., 2003, Volek et al., 2004).

These discrepancies in findings could be explained by levels of physical activity. These studies demonstrating a beneficial effect of creatine on muscle mass used supplementation alongside a training program, or used highly resistance-trained male athletes (Becque et al., 2000). Although previous studies found no association between creatine intake and lean mass in trained females (Ferguson and Syrotuik, 2006, Larson-Meyer et al., 2000), it has been suggested (Kresta et al., 2014) that there may be gender

differences in response to creatine supplementation. Although this is not categorically proven in the literature (Branch, 2003), females generally have a lower muscle mass and so would have a lower creatine uptake and consequently a smaller response to supplementation than their male counterparts. It has been suggested that vigorous muscle contraction may be necessary for the maximal uptake of creatine in healthy muscle (Robinson et al., 1999), as well as in sarcopenic and dystrophic muscle (Brose et al., 2003, Zange et al., 2002).

Present findings demonstrate that there was no significant correlation between dietary creatine intake and muscle mass. This is supported by previous findings that there is no effect on muscular strength following creatine supplementation in untrained male adults (Francaux and Poortmans, 1999) or in trained female adults (Ferguson and Syrotuik, 2006). Findings have also shown that there is no effect of creatine supplementation on muscular strength in middle-aged and older adults (Bemben et al., 2010), or in individuals suffering from myotonic dystrophy (Tarnopolsky et al., 2004a). Given the reported correlation between muscle mass and muscle strength, these findings are consistent with the lack of association shown between creatine intake and muscle mass.

In comparison, previous findings have shown that there is a positive correlation between creatine intake and muscle strength. Creatine supplementation has been shown to be associated with increases in muscular strength in trained males (Becque et al., 2000, Kreider et al., 1998a, Noonan et al., 1998, Pearson, 1999, Peeters et al., 1999) and untrained males (del Favero et al., 2012) as well as in trained females (Larson-Meyer et al., 2000). Older (mean age 68 years) and younger (mean age 22 years) adults experience increases in muscular strength following creatine supplementation (Brose et al., 2003, Cornish et al., 2009, Tarnopolsky, 2007). Creatine supplementation is also associated with increases in muscular strength in individuals suffering from some forms of muscular dystrophy and neuromuscular disorders (Tarnopolsky et al., 2004b, Tarnopolsky and Martin, 1999).

The lack of correlation demonstrated in these findings could be due to insufficient exercise stimuli to promote the creatine uptake and subsequent influence on muscle. It is also possible that the amount of creatine consumed through dietary sources is insufficient to promote measurable changes in muscular strength, particularly in the absence of sufficient exercise stimuli. The present study has demonstrated no

correlation between creatine intake and muscle mass, which supports this finding of no association between creatine intake and muscular strength.

The current study demonstrated that there was significant positive correlation between BMD of the neck of the femur, the total hip, and the lumbar spine, and; lean mass of the upper limbs, lower limbs, trunk, and subtotal whole body. In support of these results, previous work has shown that lean mass is positively correlated with BMD in young and older women (Arden and Spector, 1997, Henderson et al., 1995, Madsen et al., 1998, Mikkola et al., 2009, Pasco et al., 2015, Seeman et al., 1996, Wang et al., 2005), in premenarcheal girls (Morris et al., 1997), and in women suffering from breast cancer (Hojan et al., 2013). Lean mass is also positively correlated with BMD in adult males (Ahedi et al., 2014, Bevier et al., 1989, Travison et al., 2008).

In contrast to the current findings, lean mass is not correlated with bone strength in female gymnasts (Burt et al., 2012). Sarcopenia, the loss of muscle mass, has not been found to be associated with BMD in osteopenic or osteoporotic females (Walsh et al., 2006), although 50% of osteoporotic women also had sarcopenia. On the other hand, sarcopenia has been shown to be associated with low BMD in middle-aged and elderly men and women (Lima et al., 2009, Pereira et al., 2015, Verschueren et al., 2013).

The lack of correlation in the above studies could be explained by the population being examined. The study by Burt et al. (Burt et al., 2012) investigated the relationship between bone strength and muscle mass in gymnasts undergoing high or low levels of exercise. It is possible that the level of exercise in the “low intensity” group was sufficient to influence bone, so that the additional effect of “high intensity” exercise did not promote additional changes in bone. Walsh et al. (Walsh et al., 2006) investigated osteopenic and osteoporotic women; in these groups, the bone turnover is disturbed, and may be influenced by other factors which negated the effects of muscle mass, such as oestrogen levels. Despite this, the present study demonstrates association between muscle mass and BMD in both premenopausal and postmenopausal women. In combination with previous findings (Lima et al., 2009), this suggests that there may have been confounding factors in the study conducted by Walsh et al (Walsh et al., 2006) which resulted in no correlation being found.

Findings of the present study demonstrate that significant positive correlation was established between BMD of the hip and muscle strength of the quadriceps. These results support previous findings that muscular strength is positively correlated with BMD in healthy young girls (Henderson et al., 1995), in premenopausal and in postmenopausal women (Arden and Spector, 1997, Calmels et al., 1995, Dornemann, 1997, Hughes et al., 1995, Pasco et al., 2015, Pocock et al., 1989, Rhodes et al., 2000, Sinaki and Offord, 1988, Snow-Harter et al., 1990). BMD is positively correlated with muscular strength in active teenage and adult females (Helge and Kanstrup, 2002, Taaffe and Marcus, 2004) and in older adult males (Ahedi et al., 2014). Given the current study's reported correlation between muscle mass and muscular strength, this reported correlation between BMD and muscle strength is consistent with the association found between BMD and muscle mass.

In comparison to the present study, previous findings have failed to find a relationship between BMD and muscular strength in college-aged females (Madsen et al., 1998), college-aged gymnasts (Taaffe and Marcus, 2004), or in adult men (Hughes et al., 1995). BMD has been shown not to be correlated with muscular strength except at the proximal femur in adult females (Seeman et al., 1996), suggesting that the association may be site-specific; this is supported by Wolff's Law, as adaptations in the bone occur in response to increased loading.

The lack of correlation found in the above studies could be explained by differences in the populations investigated. Taaffe et al. (Taaffe and Marcus, 2004) found that there was positive correlation between muscular strength and BMD in healthy control participants, but not in gymnasts. It was therefore suggested that the high levels of mechanical loading undergone by gymnasts led to dissociation of the bone-muscle relationship. Given the association between body mass and BMD, it is also possible that gymnasts, with low body mass, would have a resultant low BMD. Madsen et al. (Madsen et al., 1998) found that there was no correlation between BMD and muscular strength in college-aged women. The small sample sizes used in this study would mean that there could be insufficient statistical power to reveal correlations. Given the homogeneity of the participants used, there may not have been enough natural variation in the sample to reveal statistically significant correlations in a small cohort. Hughes et al. (Hughes et al., 1995) found no significant correlation between muscular strength and

BMD in men, although significant correlation was identified in women. This study used middle-aged to older adults (45-77 years); at this stage of life, the bone metabolic responses of men and women are different. It is therefore possible that the levels of activity habitually undergone were sufficient to affect muscle strength in the men, without being of a high enough intensity to affect bone. It could also be that decreasing levels of physical activity in male participants were sufficient to decrease muscular strength, but that the decrease in bone density was happening at a slower rate; at this stage of life, loss of bone mass happens at a higher rate in women than in men.

Findings of the present study demonstrate that there was significant positive correlation between quadriceps strength and lean mass of; the upper limbs, the lower limbs, the trunk, and subtotal whole body. Much of the current literature demonstrate similar findings. Adult females exhibit significant positive correlation between lean mass and muscular strength (Arden and Spector, 1997, Seeman et al., 1996). There was significant positive correlation between lean mass and muscular strength in young, healthy adults, both male and female (Ikai and Fukunaga, 1968, Maughan et al., 1983, Schantz et al., 1983). Middle-aged and older adults also demonstrate positive correlation between lean mass and muscular strength (Ahedi et al., 2014, Arokoski et al., 2002, Bembien et al., 2010, Frontera et al., 1991).

This correlation between muscle mass and strength is site-specific; even within the hip joint, only specific muscles are correlated with leg strength (Ahedi et al., 2014). In addition to being site-specific, this correlation is action-specific. Eccentric muscle training results in muscle hypertrophy and increases in eccentric muscle strength, but does not result in increases in concentric strength of the trained muscle. Likewise, concentric muscle training results in muscle hypertrophy and increases in concentric strength, but does not result in increases in eccentric strength of the trained muscle (Higbie et al., 1996).

Despite this site-specificity in the relationship between muscle mass and strength, the present findings showed correlation between leg strength and measures of lean mass in the upper body as well as in the lower limbs. However, strength of the lower limbs is positively correlated with strength of the upper limbs (Arden and Spector, 1997, Herman et al., 2005); this would therefore explain the observed association in the present study.

The reliability of iPAQ for measuring levels of physical activity has been established in various different populations (Craig et al., 2003, Kurtze et al., 2008, Medina et al., 2013, Papathanasiou et al., 2009). Present findings did not demonstrate that there was significant correlation between habitual physical activity levels and muscle measures. This lack of correlation between reported habitual physical activity levels and muscle supports the supposition that physical activity levels in the current population were not high enough to promote changes in muscle or in bone. It is therefore possible that the low levels of physical activity were insufficient to stimulate maximal uptake and utilisation of the creatine obtained from the diet, which could explain why there was no observed relationship of creatine intake with any measures of muscle or bone.

6.5.1 Conclusion

In conclusion, habitual creatine intake is not associated with BMD, or lean mass or strength in adult women. Lean mass and muscle strength both show association with BMD. It is possible that dietary creatine intake and habitual levels of physical activity in a non-athletic population are insufficient to promote the effects of creatine on bone and muscle. Future studies will therefore aim to investigate the effects of creatine supplementation, and the effects of exercise, on BMD and muscle in adult women.

7 Study 4: The Effects of Creatine Monohydrate Supplementation on Musculoskeletal Health in Women

Having assessed the relationships between creatine intake, physical activity, BMD, and muscle in the previous study, the present study now sets out to examine the effects of supplementary dietary creatine on musculoskeletal health. The present study sets out to achieve Objective 4, to determine the influence of creatine supplementation on bone health and fat-free mass in adult women, as laid down in the Introduction (2.9.2). Having previously established the reliability of the DXA, the present study follows on from the preceding study by providing additional creatine above that obtained through the normal diet.

7.1 Abstract

Introduction: *Osteoporosis is a degenerative disease characterised by low bone mineral density (BMD), which affects 1 in 2 women over the age of 50 (National Osteoporosis Society). Creatine is an organic nitrogenous compound, naturally found in the diet, and can be synthesised in the body from amino acids. Supplementation with creatine has been shown to have a beneficial effect on body composition in both healthy (Becque et al., 2000, Brose et al., 2003) and clinical populations (Louis et al., 2002, Tarnopolsky et al., 2004b). Creatine is predominantly used by tissues with high energy demands, and is important in the metabolism of osteoblasts. It has been suggested that creatine supplementation could have a beneficial effect on BMD in osteoporotic populations (Antolic et al., 2007, Alves et al., 2012). The current study aimed to investigate the effects of long-term, low-dose creatine supplementation on BMD in adult women.*

Methods: *Adult women (n=8) (49 ± 9 years; 75.1 ± 15.3 kg; 164.8 ± 3.7 cm) completed the study. Participants were randomly assigned to one of two groups (Creatine or placebo), and took 9 months of oral supplementation; $3\text{ g}\cdot\text{day}^{-1}$ for the duration of the study with either creatine monohydrate or micro-crystalline cellulose placebo. BMD was measured by DXA at the proximal femur and lumbar spine, and fat and lean mass were established by a whole body scan. Venous blood samples were analysed for markers of bone formation, Osteocalcin (Olc) and Alkaline Phosphatase (ALP), and*

bone resorption, N-telopeptides (NTX). One-way ANOVA was performed to establish whether there were significant differences between baseline values. Mixed between-within ANOVA was conducted in order to establish whether there were significant differences in BMD, body composition, and blood marker measures between the supplement and placebo groups across the period of supplementation. Alpha level was set at 0.05.

Results: Follow-up BMD measures were not significantly different from baseline measures after supplementation. There was no significant effect of supplement on BMD of the proximal femur ($F(1,6)=0.00$; $p=0.99$; partial eta squared=0.000), of the femoral neck ($F(1,6)=0.00$, $p=0.99$, partial eta squared=0.000), or at the lumbar spine ($F(1,7)=0.04$; $p=0.85$; partial eta squared=0.006). There was no significant effect of supplement across time on markers of bone formation (Olc $F(1,6)=0.10$, $p=0.77$, partial eta squared=0.016; ALP $F(1,4)=0.14$; $p=0.72$; partial eta squared=0.035) or bone resorption (NTX $F(1,5)=0.65$, $p=0.46$, partial eta squared=0.115). There was a significant effect of supplement across time on fat-free mass (Wilks' Lambda=0.15, $F(3,4)=7.66$, $p=0.04$). There was no significant effect of supplement across time on fat mass ($F(1,6)=2.53$, $p=0.16$, partial eta squared=0.30).

Conclusion: 9 months' creatine supplementation of $3\text{g}\cdot\text{day}^{-1}$ did not result in changes in bone turnover or bone growth. There was a significant effect of creatine supplementation on fat-free mass, even without associated increases in physical activity levels.

7.2 Introduction

Creatine is a naturally occurring nitrogenous organic acid, which is highly used in the body in energy production and is involved in energy transfer from the mitochondria. Phosphocreatine is also needed to resynthesize and maintain the levels of ATP, the body's main energy source. Creatine can increase the metabolic activity of the cells involved in bone formation (Gerber et al., 2005), and may also increase satellite cell activity as well as muscle accretion and strength (Olsen et al., 2006).

Bone is a dynamic, living tissue, which is constantly being broken down and rebuilt throughout life, defining bone growth and loss; this process is known as bone turnover. The process of bone turnover is tightly controlled within the body by various hormones. As individuals age, there is a natural change in bone turnover; after the age of approximately 40, the rate of bone resorption exceeds the rate of bone formation, leading to a loss of bone mass. As women go through the menopause, there is a change in hormone balance; in particular, there is a reduction in the level of oestrogen in the body. This leads to a greater imbalance in bone turnover, and so an increase in the rate of bone loss; women are therefore at greater risk of developing osteoporosis than their male counterparts.

Bone turnover is controlled predominantly by two main cell types, osteoclasts and osteoblasts. Osteoclasts are responsible for breaking down and absorbing old bone, and osteoblasts build and lay down new bone. When the activity of osteoclasts exceeds the activity of osteoblasts, bone loss occurs. Conversely, when the activity of osteoblasts exceeds the activity of osteoclasts, bone growth occurs. Creatine has been shown to increase the metabolic activity of osteoblast-like cells (Gerber et al., 2005). It has been suggested that an increase in osteoblast activity also leads to an increase in osteoprotegerin, a cytokine which inhibits osteoclast differentiation and a subsequent decrease in bone resorption.

The development and repair of bone is a process with a high energy demand (Gualano et al., 2010), with part of that energy coming from the creatine present in the cells. Chondrocytes and osteoblasts require high amounts of energy during mineralisation. There is a potential for creatine to increase the metabolic activity of cells involved in bone formation and to decrease markers of bone resorption (Candow and Chilibeck,

2010). It has been demonstrated (Wallimann and Hemmer, 1994) that isoforms of phosphorylcreatine kinase (PCK), which is a catalyst for the regeneration of ATP from ADP, can be found in bone cartilage during various stages of development. High levels of PCK are also related to chondrocyte hypertrophy (Gerber et al., 2005, Hobson et al., 1999), further demonstrating a link between PCK and cartilage development. In addition to this, various hormones which promote bone mass development, such as insulin growth factor and parathyroid hormone, result in increased levels of PCK activity (Somjen et al., 1985, Somjen and Kaye, 1994). It has also been found (Funanage et al., 1992, Gualano et al., 2010) that use of β -guanidinopropionic acid (GPA) results in a decrease in the levels of Cr and PCr and causes endochondral disorder. Investigation into the effect of creatine being added to osteoblast-like cell cultures resulted in an increase in the cell metabolic activity and mineralisation (Gerber et al., 2005). These findings demonstrate the role of the PCr system in the development of tissues such as bone and cartilage.

In vitro studies have demonstrated that creatine supplementation has a beneficial effect on the bone remodelling process (Gerber et al., 2008, Gerber et al., 2005). Creatine supplementation in young, growing rats resulted in a significant increase in lumbar spine BMD and femur strength, and a trend towards an increase in BMD of the distal femur (Antolic et al., 2007). In addition to this, investigation into the effects of creatine on embryonic chicken femora cell cultures revealed that the addition of PCr resulted in an increase in cell mineralisation (Sekrecka-Belniak et al., 2009). In contrast, a study into the influence of creatine supplementation on the bone mass of spontaneously hypertensive rates, an experimental model for osteoporosis, did not show any significant effect of creatine on bone mass (Antolic et al., 2007).

Individuals suffering from muscular dystrophy, a disease state associated with muscle wastage and loss of bone mass, exhibit varied results when supplemented with creatine. There is some evidence that creatine supplementation can result in decreased markers of bone resorption and increased BMD in individuals suffering from some forms of muscular dystrophy (Gualano et al., 2010, Louis et al., 2002); in contrast, no significant effect on BMC or on BMD has been found following creatine supplementation in other forms of muscular dystrophy (Tarnopolsky et al., 2004a).

The long-term effects of creatine supplementation may have the potential to influence bone health. 10-14 weeks' creatine supplementation augments the effects of exercise on muscle markers and bone resorption (Brose et al., 2003, Candow et al., 2008, Chilibeck et al., 2005), and 6 months' supplementation with creatine has been shown to enhance the effects of a resistance exercise program on lean mass and fat mass in older men (Tarnopolsky et al., 2007), although 12 months' supplementation with $1\text{g}\cdot\text{day}^{-1}$ creatine monohydrate without exercise training did not result in increases in bone growth or markers of bone growth in postmenopausal women (Lobo et al., 2015). In contrast, 12 months' creatine supplementation alongside exercise has been shown to preserve BMD of the femoral neck, and increase measures of bone strength in postmenopausal women (Chilibeck et al., 2014). Given that the average dose used by Chilibeck et al. (Chilibeck et al., 2014) was $5.7\text{g}\cdot\text{day}^{-1}$, in comparison to $1\text{g}\cdot\text{day}^{-1}$ used by Lobo et al. (Lobo et al., 2015), higher doses of creatine may be necessary to elicit the changes observed.

The present study aims to investigate the effects of long-term creatine supplementation of $3\text{g}\cdot\text{day}^{-1}$ on measures of bone health in adult women. It was hypothesised that creatine supplementation would result in increases in BMD and lean mass, and increases in bone formation markers osteocalcin (Olc) and alkaline phosphatase (ALP), and a decrease in bone resorption marker NTX.

7.3 Method

7.3.1 Participants

15 Caucasian female participants (49 ± 9 years) (mass $75.11 \pm 15.33\text{kg}$) (height $164.78 \pm 3.71\text{cm}$) (BMI 27.79 ± 6.71) initially volunteered to take part in the study. Before agreeing to take part in the study, all prospective participants were given a copy of the participant information sheet (see Appendix 20). Upon arriving at the laboratory, they were given a paper copy of the same information sheet to read through, and the study was also explained verbally. Participants had the opportunity to ask any questions they may have had about the study or about their involvement. Written informed consent was obtained from each participant prior to any test being performed. Ethical approval was granted by Aberystwyth University, in accordance with the Ethical Guidelines of the Helsinki Declaration of 1961 (revised in Fortaleza, 2013). Although 15 participants enrolled on the study, 7 dropped out during the 12 months. 2 participants left during the initial 3 months, 4 between months 3 and 9, and 1 in the final 3 months. The

distribution of participants in groups throughout the study is shown below (see Figure 7.1).

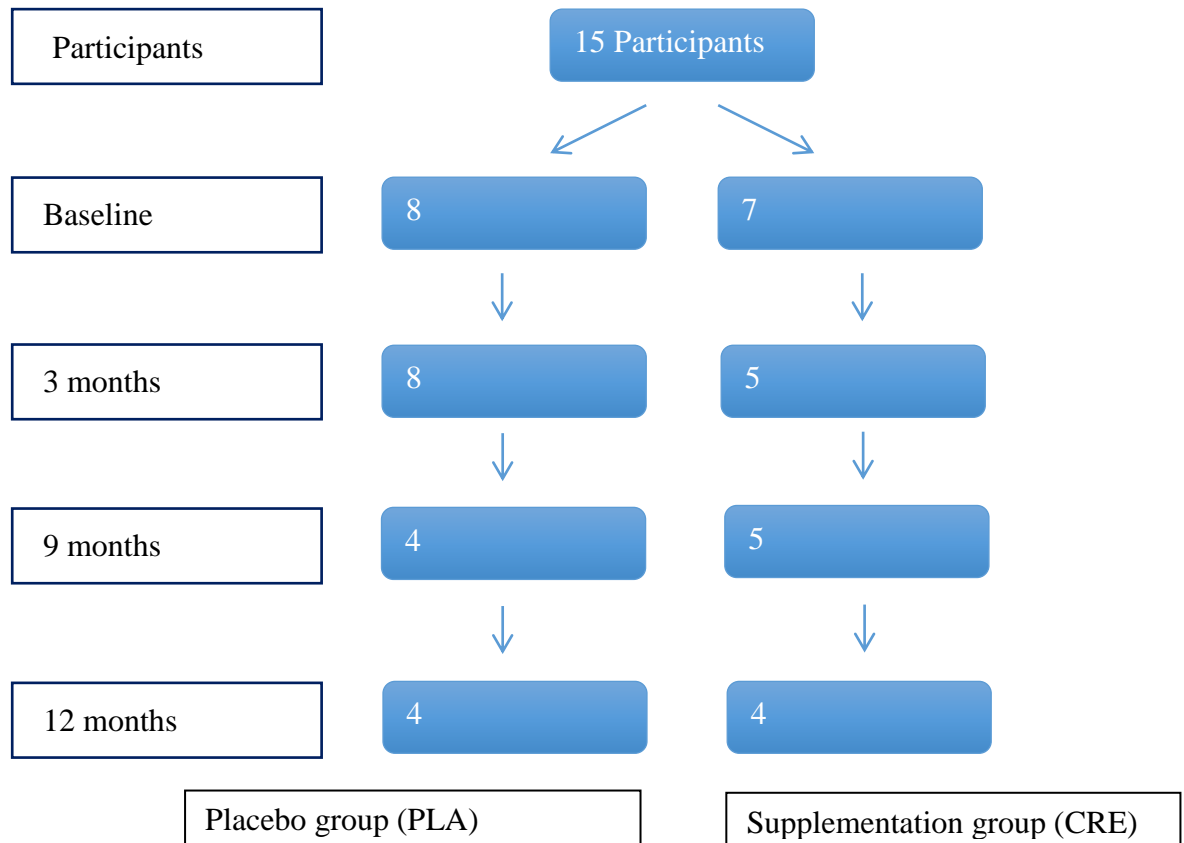


Figure 7.1: Distribution of the participants in the current study, from baseline to 12 months.

7.3.2 Study Design

The study was a double-blind design. Participants were assigned in a randomised fashion, using a block randomisation with a block size of 4, to either the supplementation group or to the placebo group. Individuals were excluded from the study if any of the following criteria were met; those currently undergoing treatment for osteoporosis, those who were or might be pregnant, those who had taken any bone-affecting drug in the 12 months previous, and those with large metal implants (e.g. hip replacement).

All measures were taken for participants at baseline, 3 months, 9 months, and 12 months. For each of these visits participants arrived after a 12 hour fast, and with a controlled fluid intake of 500ml of water that morning.

7.3.3 Protocol

7.3.3.1 Anthropometric measurements

Participants' height was measured with a stadiometer without shoes and the minimum clothes to the nearest 0.1 cm (Harpenden Stadiometer [Holtain Ltd, Crymych, UK]). A scale with 0.1 kg precision (Seca 899, Seca gmb & co, Hamburg, Germany) was used to measure participants' weight. Participant characteristics are summarised in Table 7.1. There were no significant differences in participant characteristics between groups at baseline.

7.3.3.2 Nutrition Analysis

Participants were asked to bring with them a 3-day food diary, which contained information on the type of food consumed, the amount of each food, and the way it was prepared, including 1 weekend day and 2 week days. If this was not completed prior to attending the lab, they were asked to complete a 3-day food recall whilst in the lab. Food diaries were analysed using WinDiets (WinDiets Professional 2008, The Robert Gordon University, Aberdeen) for macronutrients (fat, carbohydrate, and protein), and for calcium intake. Creatine intake was calculated from food diaries, using the creatine content of the 10 highest creatine-containing foods.

7.3.3.3 Physical Activity

Participants were also asked to complete the short version of an International Physical Activity Questionnaire (iPAQ), to establish their normal levels of physical activity. This questionnaire was repeated at each visit in order to establish any changes in habitual physical activity levels (Appendix G. iPAQ (short)).

7.3.3.4 Dual energy X-ray absorptiometry

At each of the baseline, 3 month, and 9 months visits, 3 DXA scans were conducted; the whole-body, hip, and spine. Bone area, bone mineral content, and bone mineral density were obtained via DXA scan (Hologic Discovery QDR A; APEX System

Software Version 3.4.2). Participants lay on the bed in the supine position, and proximal femur and L1-L4 spine positioning devices were used for the hip and spine scans, respectively. For the whole body scan, participants were asked to lie still in the supine position, and it was ensured that they lay within the scan area (for a more detailed procedure, see 3.3.3).

7.3.3.5 Blood Analysis

Venous blood samples were taken from each participant. All blood samples were taken by an individual trained in phlebotomy (NHS guidelines (CHS132)). Samples were taken from the medial cubital vein of the non-dominant arm. The serum was stored at -80°C, prior to analysis. All blood samples were analysed for ALP and creatinine using the Randox RX Daytona Plus (Randox Laboratories Ltd., County Antrim, UK), and for Osteocalcin using an ELISA assay kit (R&D Systems, Inc., Minneapolis, USA) and for NTX using an ELISA kit (Elabscience Biotechnology Co., Ltd., WuHan, China) (for full details of the blood collection and analysis procedure, see 3.5).

7.3.3.6 Supplementation

All participants were randomly allocated to one of two groups using a random number generator. Participants assigned to the placebo group (PLA) took a supplement of micro-crystalline cellulose (Blackburn Distributions, Lancashire, England), and those in the intervention group (CRE) took creatine monohydrate (Creapure, AlzChem, Trostberg, Germany). Both supplements were a white powder. In order to create a supplementation design which was more reflective of ‘real-life’, and which was sustainable and palatable over a long duration, a low dose supplement was used without the traditional “loading phase” often seen. Participants took 3g·day⁻¹ of their given supplement for the duration of the study. Given the habitual creatine intake of participants (see Table 7.1), this dose was a large increase in intake for all participants.

All participants were initially given 2 weeks’ worth of the supplement, and supplement tubs were re-weighed after 2 weeks and then refilled. This was then repeated after a further 2 weeks, and then monthly in order to monitor adherence. After 9 months’ supplementation, all participants returned supplement tubs. Participants were then asked to maintain their normal lifestyles for a further 3 months, which served as a

“wash-out” period. All participants achieved a minimum of 80% adherence to the supplementation protocol.

7.3.4 Analysis

All data were analysed using SPSS (SPSS for Windows, Version 21.0, SPSS Inc., Chicago). Independent sample t-tests were performed to establish whether there were significant differences between baseline values. Mixed between-within ANOVA was conducted to assess the impact of creatine supplementation on participants’ measures of proximal femur BMD, femur neck BMD, lumbar spine BMD, Olc, ALP, NTX, fat-free mass, and fat mass. Post-hoc power analyses were performed to establish the likelihood of a type II error occurring, and to calculate the number of participants required to achieve a power of 0.80.

Data for each participant were also analysed individually, to investigate whether some individuals had a greater response to the supplement. Individual responses were then compared to the LSC calculated previously (see Chapter 4). Analysing each participant in this way did not affect the overall findings of the study, and so only the results for participants as groups are presented below.

7.4 Results

There were no significant differences in baseline measures between groups, except for lumbar spine BMD ($p=0.047$) (Table 7.2); participants in the creatine group had significantly higher lumbar spine BMD than those in the control group. There was no significant difference in dietary creatine intake between groups at baseline. There was no significant difference in vitamin D intake between groups at baseline, but the creatine group consumed significantly more calcium than the control ($p=0.005$). There were no significant differences in physical activity levels between groups at baseline. There were no significant changes in physical activity levels across the intervention in either group. Calcium intake was used as a covariate for assessing change in BMD across the intervention, and baseline lumbar spine BMD was used as a covariate for assessing change in lumbar spine BMD across the intervention. Physical activity levels were used as a covariate for assessing body composition changes across the

intervention. Participants' results for BMD and body composition measures throughout the study are summarised in Table 7.2 and Table 7.3, respectively.

Table 7.1: Participant characteristics. All data are presented as mean \pm Standard deviation. CRE = creatine group, PLA = control group

	Age (years)	Height (cm)	Mass (kg)	BMI (kg·m⁻²)	Fat mass (kg)	Lean mass (kg)	Cr Intake (g)
CRE	45.3 \pm 9.1	165.7 \pm 3.2	79.26 \pm 16.96	29.13 \pm 9.20	32.39 \pm 10.76	46.86 \pm 7.68	1.93 \pm 0.71
PLA	52.3 \pm 8.7	162.9 \pm 4.5	70.89 \pm 8.49	28.07 \pm 4.94	28.11 \pm 5.59	42.78 \pm 4.60	1.87 \pm 0.49
Total	49.0 \pm 9.2	163.8 \pm 4.1	74.79 \pm 13.34	28.52 \pm 6.38	30.11 \pm 8.38	44.69 \pm 6.35	1.90 \pm 0.58

Table 7.2: BMD values at each time point. *denotes significant difference from baseline measures. † represents significant difference between creatine and control groups. Significant changes presented have been corrected for calcium intake.

	Creatine	Control
Baseline		
Femur Neck BMD ($\text{g}\cdot\text{cm}^{-2}$)	0.90 \pm 0.11	0.83 \pm 0.13
Hip BMD ($\text{g}\cdot\text{cm}^{-2}$)	1.07 \pm 0.11	0.99 \pm 0.14
Lumbar Spine ($\text{g}\cdot\text{cm}^{-2}$)	1.11 \pm 0.11	0.99 \pm 0.10 †
3 months		
Femur Neck BMD ($\text{g}\cdot\text{cm}^{-2}$)	0.89 \pm 0.13	0.82 \pm 0.14
Hip BMD ($\text{g}\cdot\text{cm}^{-2}$)	1.04 \pm 0.12	0.98 \pm 0.15
Lumbar Spine ($\text{g}\cdot\text{cm}^{-2}$)	1.09 \pm 0.14	0.97 \pm 0.10
9 months		
Femur Neck BMD ($\text{g}\cdot\text{cm}^{-2}$)	0.88 \pm 0.14	0.84 \pm 0.09
Hip BMD ($\text{g}\cdot\text{cm}^{-2}$)	1.04 \pm 0.12	1.01 \pm 0.07
Lumbar Spine ($\text{g}\cdot\text{cm}^{-2}$)	1.07 \pm 0.13	0.95 \pm 0.07
12 months		
Femur Neck BMD ($\text{g}\cdot\text{cm}^{-2}$)	0.82 \pm 0.12	0.83 \pm 0.08*
Hip BMD ($\text{g}\cdot\text{cm}^{-2}$)	1.01 \pm 0.10	1.00 \pm 0.08
Lumbar Spine ($\text{g}\cdot\text{cm}^{-2}$)	1.05 \pm 0.14	0.95 \pm 0.07

Table 7.3: Subtotal body composition values at each time point. * denotes significant difference from baseline values. Significant changes presented have been corrected for physical activity levels.

	Creatine	Control
Baseline		
Fat mass (kg)	32.39±10.76	28.11±5.59
Lean mass (kg)	46.86±7.68	42.78±4.60
Subtotal mass (kg)	79.26±16.96	70.89±8.49
3 months		
Fat mass (kg)	35.20±10.35	28.71±5.88
Lean mass (kg)	47.59±8.75	42.44±4.57
Subtotal mass (kg)	82.89±17.94	71.15±8.51
9 months		
Fat mass (kg)	29.34±6.67	22.44±0.86
Lean mass (kg)	46.54±8.96	42.08±0.97
Subtotal mass (kg)	75.88±15.19	64.52±0.75
12 months		
Fat mass (kg)	30.88±7.08	22.96±1.01
Lean mass (kg)	44.70±8.68	41.98±1.41*
Subtotal mass (kg)	75.58±15.36	64.94±0.98

7.4.1 BMD

BMD values at baseline and at 9 months, and change in BMD measures between baseline and 9 months, are presented in Table 7.4.

Table 7.4: Change in BMD values across the intervention. Given that the time period 9-12 months was a wash-out period, values between baseline and 9 months are shown. Creatine = creatine group, Control = control group, Δ = change between baseline and 9 months.

	Baseline	9 months	Δ
Creatine			
Femur Neck BMD ($\text{g}\cdot\text{cm}^{-2}$)	0.90 ± 0.11	0.88 ± 0.14	-0.05 ± 0.05
Hip BMD ($\text{g}\cdot\text{cm}^{-2}$)	1.07 ± 0.11	1.04 ± 0.18	0.00 ± 0.03
Lumbar Spine ($\text{g}\cdot\text{cm}^{-2}$)	1.11 ± 0.11	1.07 ± 0.13	0.00 ± 0.02
Control			
Femur Neck BMD ($\text{g}\cdot\text{cm}^{-2}$)	0.83 ± 0.13	0.84 ± 0.09	-0.05 ± 0.04
Hip BMD ($\text{g}\cdot\text{cm}^{-2}$)	0.99 ± 0.14	1.01 ± 0.07	-0.03 ± 0.02
Lumbar Spine ($\text{g}\cdot\text{cm}^{-2}$)	0.99 ± 0.10	0.95 ± 0.07	-0.02 ± 0.02

7.4.1.1 Femur Neck BMD

There was no significant main interaction between supplement and time on femur neck BMD ($F(3,4)=0.42$, $p=0.74$, partial eta squared=0.065). There was a significant main effect for time ($F(3,4)=7.70$, $p<0.01$, partial eta squared=0.562), with both groups showing a reduction in femur neck BMD across the four time periods (see Table 7.2). The main effect comparing the creatine supplement to the control group was not significant ($F(1,6)=0.00$, $p=0.99$, partial eta squared=0.000).

7.4.1.2 Hip BMD

There was no significant interaction between supplement and time on hip BMD ($F(3,4)=0.93$; $p=0.45$; partial eta squared=0.14). There was no significant of time on hip BMD ($F(3,4)=1.15$; $p=0.36$; partial eta squared=0.16). There was no effect of supplement on hip BMD ($F(1,6)=0.00$; $p=0.99$; partial eta squared=0.000) (see Table 7.2).

7.4.1.3 *Lumbar Spine BMD*

There was no significant interaction between supplement and time on lumbar spine BMD ($F(3,4)=0.06$; $p=0.98$; partial eta squared=0.009). There was a significant main effect for time ($F=3.81$, $p=0.03$, partial eta squared=0.389), with both groups showing a reduction in lumbar spine BMD across the four time periods (see Table 7.2). The main effect comparing the creatine supplement to the control group was not significant ($F(1,6)=1.40$; $p=0.28$; partial eta squared=0.189).

Difference in lumbar spine BMD was calculated, and ANCOVA was conducted to establish the effect of creatine supplement on change in lumbar spine BMD over time, using baseline lumbar spine BMD as a covariate. The assumption of sphericity was violated for change in lumbar spine BMD over time (Mauchly's $W=0.18$; $p=0.01$). Multivariate tests revealed that there was no significant interaction between supplement and time on change in lumbar spine BMD (Wilks' Lambda=0.97; $F(2,5)=0.09$; $p=0.92$). There was no significant effect of time on change in lumbar spine BMD (Wilks' Lambda=0.88; $F(2,5)=0.35$; $p=0.72$). There was no significant main effect of supplement on change in lumbar spine BMD ($F(1,7)=0.04$; $p=0.85$; partial eta squared=0.006).

7.4.2 **Blood Markers**

7.4.2.1 *Osteocalcin*

There was no significant interaction of supplement and time on Olc levels ($F(3,4)=2.41$, $p=0.10$, partial eta squared=0.287). There was no significant effect of time on Olc levels ($F(3,4)=3.05$, $p=0.06$, partial eta squared=0.337). There was no significant main effect of supplement on Olc levels ($F(1,6)=0.10$, $p=0.77$, partial eta squared=0.016) (see Figure 7.2).

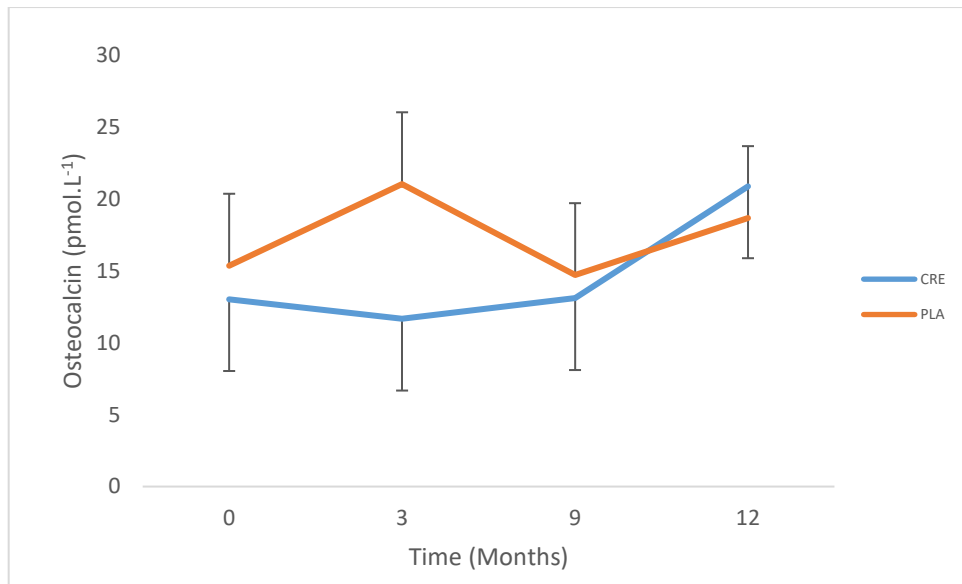


Figure 7.2: Osteocalcin per group across time (baseline, 3 months, 9 months, 12 months). Blue = creatine group, orange = control group.

7.4.2.2 Alkaline Phosphatase

There was no significant interaction of supplement and time on ALP levels ($F(3,2)=2.51$; $p=0.11$; partial eta squared=0.386). There was no significant effect of time on ALP levels ($F(3,2)=1.46$; $p=0.27$; partial eta squared=0.268). There was no significant main effect of supplement on ALP levels ($F(1,4)=0.14$; $p=0.72$; partial eta squared=0.035) (see Figure 7.3).

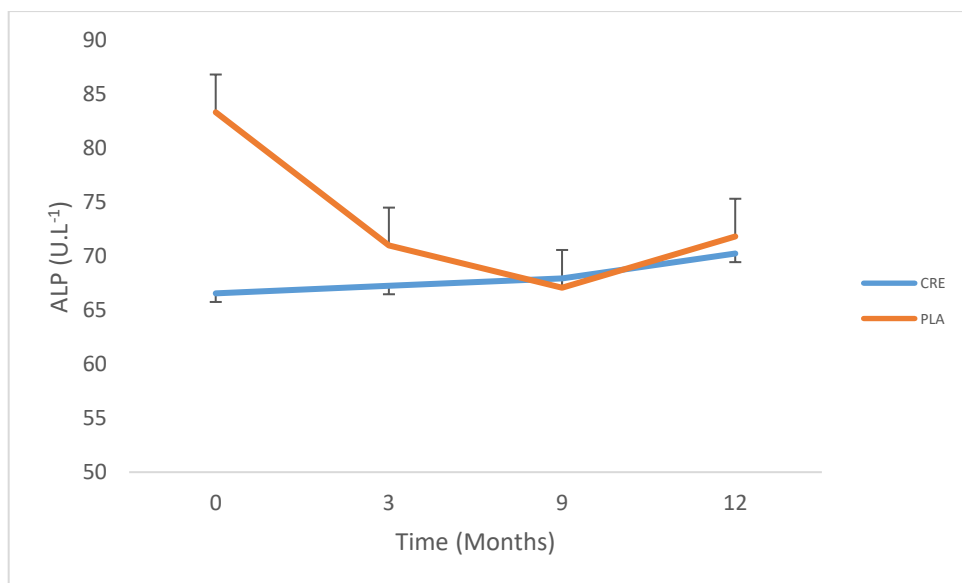


Figure 7.3: ALP per group across time (baseline, 3 months, 9 months, 12 months). Blue = creatine group, orange = control group.

7.4.2.3 *N-Telopeptides*

There was no significant overall interaction of supplement and time on NTX levels ($F(3,3)=2.17$; $p=0.13$, partial eta squared=0.303). There was no significant effect of time on NTX levels ($F(3,3)=1.01$, $p=0.42$, partial eta squared=0.168). There was no significant main effect of supplement on NTX levels ($F(1,5)=0.65$, $p=0.46$, partial eta squared=0.115) (see Figure 7.4).

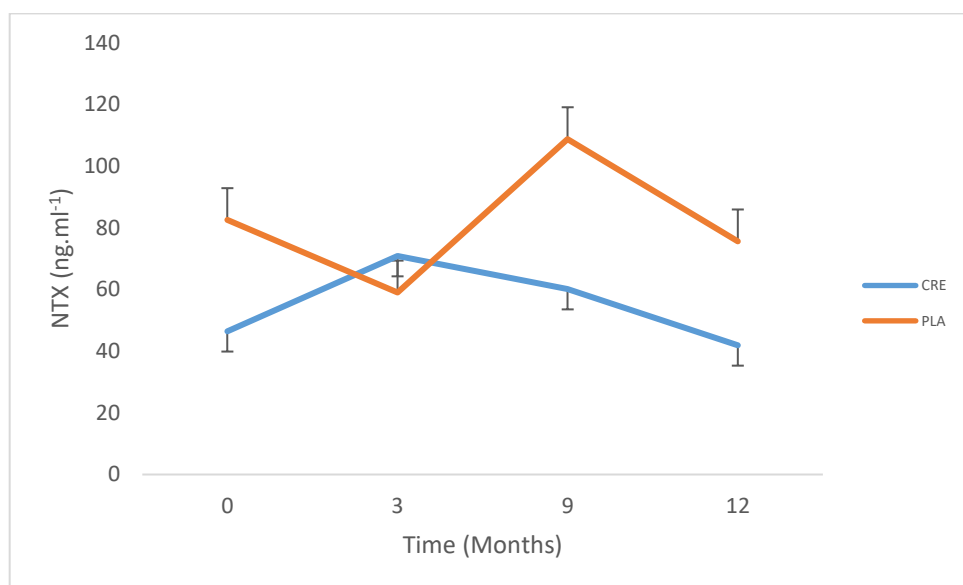


Figure 7.4: NTX per group across time (baseline, 3 months, 9 months, 12 months). Blue = creatine group, orange = control group.

7.4.3 **Body Composition**

Change in body composition measures between baseline and 9 months are presented in Table 7.5.

Table 7.5: Change in body composition values across the intervention. Given that the time period 9-12 months was a wash-out period, values between baseline and 9 months are shown. Creatine = creatine group, control = control group. % Δ = percentage change between baseline and 9 months.

	Baseline	9 months	% Δ
Creatine			
Upper limb fat mass	3.49 \pm 0.87	3.35 \pm 0.72	-3.28 \pm 1.97
Upper limb fat-free mass	4.55 \pm 0.69	4.41 \pm 0.75	-1.59 \pm 6.46
Upper limb % fat	43.12 \pm 3.96	43.01 \pm 2.50	-0.84 \pm 4.03
Lower limb fat mass	12.94 \pm 4.66	11.17 \pm 3.13	0.37 \pm 5.27
Lower limb fat-free mass	16.56 \pm 3.24	16.03 \pm 3.59	1.47 \pm 5.18
Lower limb % fat	42.98 \pm 5.24	40.82 \pm 2.15	-0.63 \pm 4.35
Subtotal fat mass	32.39 \pm 10.76	29.34 \pm 6.67	-0.63 \pm 5.73
Subtotal fat-free mass	46.86 \pm 7.68	46.54 \pm 8.96	2.16 \pm 4.55
Subtotal % fat	40.13 \pm 5.65	38.54 \pm 3.00	-1.61 \pm 5.16
Control			
Upper limb fat mass	3.31 \pm 0.47	2.69 \pm 0.16	-10.47 \pm 6.13
Upper limb fat-free mass	4.23 \pm 0.62	4.36 \pm 0.38	-2.81 \pm 7.25
Upper limb % fat	44.04 \pm 5.44	38.25 \pm 2.08	-4.70 \pm 6.97
Lower limb fat mass	10.57 \pm 2.26	8.87 \pm 1.57	-11.14 \pm 11.57
Lower limb fat-free mass	14.50 \pm 1.78	14.31 \pm 0.51	-3.89 \pm 4.01
Lower limb % fat	41.90 \pm 5.53	38.04 \pm 4.08	-4.48 \pm 5.65
Subtotal fat mass	28.11 \pm 5.59	22.44 \pm 0.86	-12.83 \pm 13.27
Subtotal fat-free mass	42.78 \pm 4.60	42.08 \pm 0.97	-4.26 \pm 3.28
Subtotal % fat	39.48 \pm 4.40	34.78 \pm 1.27	-5.91 \pm 7.66

7.4.3.1 *Fat-free mass*

The assumption of sphericity was violated for upper limb fat-free mass (Mauchly's $W=0.08$, $p=0.04$). Multivariate tests revealed that there was no significant interaction between time and supplement on upper limb fat-free mass (Wilks' Lambda=0.79, $F(3,4)=0.34$, $p=0.79$). There was a substantial main effect for time on upper limb fat-free mass (Wilks' Lambda=0.05, $F(3,4)=27.25$, $p=0.004$), with both groups showing a reduction in upper limb fat-free mass across the four time periods. The main effect comparing the supplement to control was not significant ($F(1,6)=0.03$, $p=0.87$, partial eta squared=0.005).

There was no significant interaction between time and supplement on lower limb fat-free mass ($F(3,4)=1.23$, $p=0.33$, partial eta squared=0.17). There was a substantial main effect of time ($F(3,4)=3.76$, $p=0.03$, partial eta squared=0.385), with both groups showing a reduction in lower limb fat-free mass across the four time periods. The main effect comparing the supplement to control was not significant ($F(1,6)=0.58$, $p=0.48$, partial eta squared=0.088).

The assumption of sphericity was violated for subtotal fat-free mass (Mauchly's $W=0.08$, $p=0.04$). Multivariate tests revealed that there was a significant interaction between time and supplement on subtotal fat-free mass (Wilks' Lambda=0.15, $F(3,4)=7.66$, $p=0.04$), with the creatine group experiencing an increase in fat-free mass during supplementation, and the control group experiencing a decrease in fat-free mass (see Figure 7.5). This change exceeded the LSC calculated in Study 1 (see 4.4).

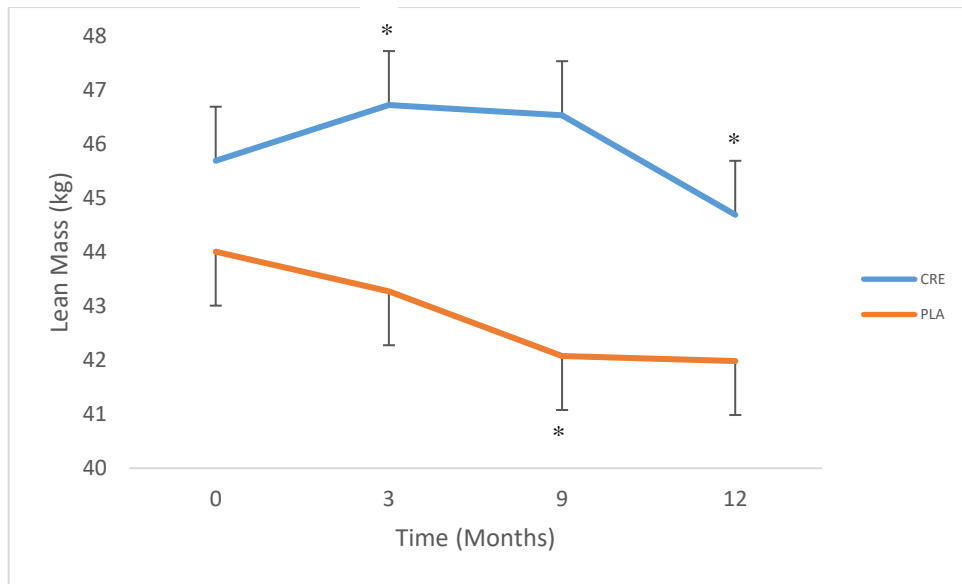


Figure 7.5: Lean mass per group across time (baseline, 3 months, 9 months, 12 months). Blue = creatine group, orange = control group.

7.4.3.2 Fat Mass

There was no significant interaction between time and supplement on upper limb fat mass ($F(3,4)=1.74$, $p=0.20$, partial eta squared=0.224). There was a substantial main effect for time ($F(3,4)=5.11$, $p=0.010$, partial eta squared=0.460), with both groups exhibiting an increase in upper limb fat mass between baseline and 3 months, and between 9 and 12 months. Both groups exhibited a decrease in fat mass between 3 months and 9 months. The main effect comparing the supplement to control was not significant ($F(1,6)=2.55$, $p=0.16$, partial eta squared=0.298).

The assumption of sphericity was violated for lower limb fat mass (Mauchly's $W=0.07$, $p=0.03$). Multivariate tests revealed that there was no significant interaction between time and supplement on lower limb fat mass (Wilks' Lambda=0.54, $F(3,4)=1.15$, $p=0.43$). There was a substantial effect for time (Wilks' Lambda=0.15, $F(3,4)=7.13$, $p=0.04$), with all groups exhibiting a decrease in fat mass between 3 months and 9 months, and an increase in fat mass between 9 months and 12 months. The main effect comparing the supplement to control was not significant ($F(1,6)=0.96$, $p=0.37$, partial eta squared=0.14).

The assumption of sphericity was violated for subtotal fat mass (Mauchly's $W=0.06$, $p=0.02$). Multivariate tests revealed that there was no significant interaction between time and supplement on subtotal fat mass (Wilks' $\Lambda=0.54$, $F(3,4)=1.13$, $p=0.44$). There was a substantial effect for time (Wilks' $\Lambda=0.15$, $F(3,4)=7.48$, $p=0.04$), with all groups exhibiting a decrease in fat mass between 3 months and 9 months, and an increase in fat mass between 9 months and 12 months. The main effect comparing supplement to control was not significant ($F(1,6)=2.53$, $p=0.16$, partial $\eta^2=0.30$).

Given the reports of “responders” and “non-responders” to creatine supplementation, it was possible that “non-responders” were clouding the data, masking the effects of supplementation on “responders”. Therefore, data for each participant was analysed individually. BMD and body composition changes were then compared to LSC calculated previously (see Chapter 4). No significant relationships of creatine intake with BMD or bone turnover markers were observed following creatine supplementation (all $p > 0.05$), and no body composition changes other than those reported above for each group were observed (all $p > 0.05$).

7.5 Discussion

The present study aimed to investigate the effects of a long-term, low-dose creatine supplementation on BMD in adult women. It was hypothesised that creatine supplementation would result in increases in BMD, increased markers of bone formation, and decreased bone resorption in comparison to control. Results of the present study do not support the hypothesis; no significant effect of creatine supplementation on BMD or on bone turnover was observed. It was further hypothesised that creatine supplementation would result increased fat-free mass in comparison to control. In support of this hypothesis, creatine supplementation was shown to result in increased fat-free mass in comparison to control.

Bone loss in adults naturally occurs as a result of disturbances in bone turnover, when the rate of bone resorption exceeds the rate of bone formation (see 2.1.6). In the present study, there was no effect of creatine supplementation on bone resorption (NTX) or on bone formation (Olc and ALP). In support of the present findings, creatine supplementation has been shown to have no effect on bone turnover in older adults

undergoing resistance training (Brose et al., 2003, Gualano et al., 2014b, Tarnopolsky et al., 2007), or in some rodent models of osteoporosis (Alves et al., 2012, Murai et al., 2015).

Studies investigating the effects of creatine supplementation in muscular dystrophy have shown discrepant results. Creatine supplementation has been shown to increase muscle mass and strength in a number of muscular disorders (Tarnopolsky and Martin, 1999). Supplementation with creatine results in increases in BMD, and decreases in markers of bone breakdown, in some forms of muscular dystrophy (Tarnopolsky et al., 2004b, Louis et al., 2002) although not in all forms of muscular dystrophy (Tarnopolsky et al., 2004a); given findings that creatine supplementation may also influence muscle mass and strength in some, but not all, forms of muscular dystrophy (Kley et al., 2013), it can be seen that findings regarding therapeutic effects of creatine cannot necessarily be extrapolated to other disease states.

The present study demonstrated that a low dose of creatine is not sufficient to elicit changes in bone metabolism. In support of these findings, previous work has demonstrated that lower doses of $1\text{g}\cdot\text{day}^{-1}$ for 12 months does not result in measurable changes in BMD in postmenopausal women (Lobo et al., 2015). Supplementation with $3\text{g}\cdot\text{day}^{-1}$ for 12 months also results in no change in BMD in postmenopausal women (Gualano et al., 2014b). Given that the response of adult bone is different from young bone, and that the same stimuli is less effective at increasing BMD in individuals with low bone mass (Kish et al., 2015, Razi et al., 2015), postmenopausal women, with already lower bone mass, may require a greater stimuli to elicit changes comparable to those seen in their premenopausal counterparts.

In support of the present findings, creatine supplementation with exercise has been shown to have no effect on bone turnover (Brose et al., 2003, Gualano et al., 2014b, Tarnopolsky et al., 2007). Studies investigating the combination of creatine supplementation and exercise have been varied; the studies which found no effect of supplementation on markers of bone turnover used only strength-based exercises, and no impact exercises (Brose et al., 2003, Tarnopolsky et al., 2007), or used postmenopausal women, a population with disordered bone turnover (Gualano et al., 2014b). Given the suggestion that increases in bone occur as a result of increased muscle mass (Chilibeck et al., 2005), it is possible that over only 3-6 months, more

intense exercise is necessary to promote changes in bone metabolism. Exercise is a less effective intervention in conditions with low bone mass (Nikander et al., 2010a), therefore postmenopausal women may have already experienced too much bone loss for only 4 months of exercise to significantly affect this loss.

In vitro studies have demonstrated that creatine supplementation results in increases in measures of ALP, and in increased activity and mineralisation of osteoblast-like cells (Gerber et al., 2005); this leads to increased OPG secretion, which decreases the production of osteoclasts and so reduces bone breakdown (Gerber et al., 2008). *In vivo*, changes in markers of bone turnover in favour of bone formation have been shown as a result of creatine supplementation in healthy bone (Candow et al., 2008, Cornish et al., 2009) as well as in *in vitro* experimental models of osteoporosis (Antolic et al., 2007, de Souza et al., 2012).

The present findings of no change in bone turnover following creatine supplementation were paralleled by findings in BMD; there was no effect of creatine supplementation on measures of BMD in comparison to control. All participants experienced a decrease in BMD across time, consistent with expected bone changes in this population. Given the low dose supplementation regime used in the present study, higher doses may result in changes in bone turnover. It is also possible that there was not sufficient stimulus in participants of the present study to stimulate uptake and use of the creatine; supplementation with creatine during exercise training in older men and women results in increases in BMD greater than training alone (Chilibeck et al., 2005, Chilibeck et al., 2014).

The present findings suggest that creatine supplementation has no effect against the bone loss which happens as a part of ageing. Significant losses in BMD were seen in both the femur neck and the lumbar spine, however there was a trend towards a decrease in BMD of the total hip in the control group, which was not seen in individuals supplementing with creatine. Given the small sample sizes used in the present study, it is possible that a larger sample size would have resulted in statistically significant changes in hip BMD. Power calculations show that 36 participants would be required to demonstrate significance in hip BMD at a power level of 0.8.

The creatine supplement was consumed between baseline and 9 months, followed by a 3-month washout period. After cessation of the supplement period, values began to return to baseline. Generally, a 4-6 week washout period has been adopted for use in creatine supplementation studies; the present findings demonstrate that this appears to be long enough for measures of fat-free mass to return to baseline despite the long supplementation duration, and that a longer wash-out period is not necessary.

No significant changes were observed in BMD, possibly due to the duration of supplementation; given that it takes a minimum of 6-9 months for measurable changes in BMD to occur, longer duration supplementation may result in greater changes in BMD. However, the feasibility of longer duration supplementation is uncertain, given the low adherence to the present study; adherence was good at 3 months, but poor at 9 months. Similarly, long-term studies investigating supplementation with calcium and vitamin D show high drop-out rates, between 20 and 30% in 12 months (Dawson-Hughes et al., 1997, Elders et al., 1994), and those investigating 12 months of creatine supplementation also demonstrate drop-out rates of between 20 and 25% (Gualano et al., 2014b, Lobo et al., 2015). Although these drop-out rates are not as high as those seen in the present study, long duration studies should therefore aim to over-recruit, to allow for this high drop-out rate; longer-term interventions may have limited feasibility due to the high loss of participants.

In comparison to the present findings in creatine supplementation, calcium supplementation has been shown to result in increases in bone density (Dawson-Hughes et al., 1997); however, the average length of trials investigating calcium supplementation is 3.5 years (Tang et al., 2007) in comparison to the 12 months of the present study. A longer duration supplement intervention may therefore result in changes which are not apparent after only 9 months' supplementation. 1 years' supplementation with calcium has been shown to have a significant protective effect against bone loss, although there is disagreement over the site-specificity of the effect; a protective effect has been shown in the whole body with no change in the femoral neck or the lumbar spine (Dawson-Hughes et al., 1997), and conversely in the lumbar spine (Elders et al., 1994). Vitamin D supplementation in combination with calcium has been shown to result in increased femoral neck BMD (Ooms et al., 1995) and spine BMD (Dawson-Hughes et al., 1991) after 1 year in comparison to calcium alone,

although it has been suggested treatment of calcium with or without vitamin D is similarly effective (Tang et al., 2007). There is a paucity of studies investigating the effects of vitamin D supplementation without concurrent calcium supplementation. The effect of calcium supplementation is limited; it reduces bone loss, but does not prevent loss altogether (Dawson-Hughes, 1991, Elders et al., 1994, Smith et al., 1989b).

Sarcopenia, loss of muscle mass and function (Cruz-Jentoft et al., 2010), happens as individuals age. It is often accompanied by loss of bone mass (Crepaldi and Maggi, 2005). Participants in the placebo group showed a significant decrease in lean mass over the duration of the study. There was no effect on fat mass. In comparison, the creatine group showed no decrease in lean mass; physical activity levels reported did not change significantly across the period of the intervention for either the creatine or the control group, suggesting that there was a protective effect of creatine supplementation against muscle loss.

In support of the present findings, the addition of creatine supplementation results in increased gains in lean mass than exercise without supplementation in healthy older men and women, and in younger men (Aguilar et al., 2013, Brose et al., 2003, Candow et al., 2008, Chrusch, 2001, Parise, 2001, Tarnopolsky et al., 2007). Creatine supplementation alongside resistance training also results in increased lean mass in postmenopausal women (Neves et al., 2011) and in patients suffering from Parkinson's Disease (Hass et al., 2007) or muscular dystrophy (Tarnopolsky et al., 2004b).

In contrast to these findings, creatine supplementation in addition to exercise training has been shown to have no effect on lean mass on middle-aged and older men (Bemben et al., 2010, Eijnde et al., 2003). However, there is some difference in the protocol used; the study by Eijnde et al. (Eijnde et al., 2003) did not use resistance training in the exercise protocol. Previous work has suggested that Bemben et al. (Bemben et al., 2010) did not have statistical power in the statistical tests used for full comparison (Candow et al., 2014a).

The current results suggest that creatine supplementation may provide a protective effect against muscle loss. It is possible that the protection of muscle may ultimately result in protection of bone, in line with Wolff's Law and the Mechanostat theory (Schoenau, 2005). There was no significant correlation between change in lean mass

and change in BMD at any site, however there was significant correlation between change in Osteocalcin and change in lean mass ($r=0.839$, $p=0.009$). This suggests that, although lean mass may not be directly responsible for protecting against loss of bone mass, there are similar factors which influence both bone and muscle growth and loss. Participants in the present study did not demonstrate any significant change in habitual physical activity levels which could have explained the current findings of the effect of creatine supplementation on bone formation.

The present study was limited by inclusion of only a small number of participants, resulting in low statistical power for the statistical tests performed ($P=0.33$). Power calculations revealed that a much larger number of participants would be necessary in order to achieve sufficient power; 94 participants would be required, split evenly between the groups. The present study is therefore underpowered, and so conclusions from the present study cannot necessarily be applied to the general population. This study therefore provides pilot data suggesting that creatine supplementation may have the potential to guard against loss of muscle mass.

7.5.1 Conclusion

Findings of the present study suggest that creatine supplementation may provide a protective effect against muscle wastage, although not against bone loss, in adult women. This study provides pilot data, and therefore a future study should investigate the effects of creatine supplementation on muscle loss in a much larger cohort. Muscle loss is correlated with, but has not been conclusively shown to have, a protective effect on bone. Supplementation alone is not sufficient to result in increases in bone formation. In the light of previous studies which have demonstrated a therapeutic effect of creatine on bone following resistance exercise, future studies should examine the effect of creatine supplementation in combination with exercise on BMD in adult women.

8 Study 5: The Effects of Creatine Monohydrate Supplementation and Exercise on Musculoskeletal Health in Women

The final experimental chapter in this thesis now sets out to achieve Objective 5, to design and evaluate an intervention to investigate the effects of high impact exercise augmented by creatine on musculoskeletal health in adult women (see 2.9.2). Having previously investigated the effects of supplementation with creatine monohydrate, the present study draws on these findings and moves on to investigate the effects of an exercise program and creatine supplementation on musculoskeletal health. This study follows on from the findings of the previous studies, relying on the reliability of the DXA throughout the period of supplementation, and building on the findings of the two preceding studies.

8.1 Abstract

Introduction: *Individuals who have a greater peak bone mass before undergoing the menopause are at lower risk for developing osteoporosis. Creatine supplementation may result in increases in fat-free mass (Kreider et al., 1998a), with subsequent increases in forces for bone loading, due to increased pull of muscle on bone at sites of attachment. Resistance training in combination with creatine supplementation has shown the potential to preserve BMD and decrease markers of bone resorption in older men and in postmenopausal women (Candow et al., 2008, Chilibeck et al., 2005, Chilibeck et al., 2014). The present study aims to investigate the effects of creatine supplementation and exercise on BMD, body composition, and strength in adult women.*

Methods: *Thirty-eight adult females (Age = 44.2 ± 9.5 years, Height = 1.66 ± 0.06 m, Mass = 73.3 ± 14.0 kg, BMI = 26.6 ± 5.0 kg·m⁻²) took part in this study. Participants were randomly assigned to one of four groups; high impact and resistance exercise plus creatine (n=10), high impact and resistance exercise with placebo (n=9), non-exercise with creatine (n=10), and non-exercise with placebo (n=9). Participants in the exercise group underwent 12 weeks' exercise training twice per week, during which all participants underwent 12 weeks' supplementation with 3g·day⁻¹ creatine monohydrate or placebo. All measures were completed for participants before and after the*

intervention. Measures included; BMD and body composition by DXA, isokinetic leg strength at 60deg·sec⁻¹ and at 120deg·sec⁻¹ by Biodex, Osteocalcin, Alkaline Phosphatase, and N-telopeptides, and fitness measures. Fitness measures included back strength, handgrip strength, jump height, 50m walk speed, stand-and-sit, balance, and sit-and-reach. Data was analysed using SPSS for Windows. Mixed between-within ANOVA was conducted to establish interaction between creatine and exercise across time for all measures. Alpha level was set at 0.05.

Results: *There was no significant main effect comparing the interaction of supplement and exercise on femoral neck BMD ($F(3,33)=1.31$, $p=0.26$, partial eta squared=0.038), on proximal femur BMD ($F(3,33)=1.65$, $p=0.21$, partial eta squared=0.048), or on lumbar spine BMD ($F(3,34)=1.72$, $p=0.20$, partial eta squared=0.048). There was no significant interaction between supplementation and exercise on Olc ($F(3,34)=0.50$, $p=0.48$, partial eta squared=0.015), on ALP ($F(3,34)=0.05$, $p=0.83$, partial eta squared=0.001), or on NTX:ALP ratio ($F(3,34)=3.82$, $p=0.06$, partial eta squared=0.101). There was a significant interaction of exercise and time on upper limb fat-free mass ($F(1,34)=5.40$, $p=0.03$, partial eta squared=0.137), with exercise resulting in increased upper limb fat-free mass. There was a significant interaction between time and exercise on subtotal fat mass ($F(1,34)=4.50$, $p=0.04$, partial eta squared=0.117), with decreased fat mass following exercise. There was a significant interaction of time and supplement on torque at 60 deg·sec⁻¹ ($F(1,30)=6.22$, $p=0.02$, partial eta squared=0.172), and at 120 deg·sec⁻¹ ($F(1,30)=4.11$, $p=0.05$, partial eta squared=0.121), with increased strength following creatine supplementation.*

Conclusion: *Short duration, low dose creatine supplementation during exercise training does not result in measurable changes in BMD or body composition in adult women. However, creatine supplementation does result in strength gains regardless of exercise. Exercise results in increased fat-free mass and decreased fat mass regardless of creatine supplementation in adult women.*

8.2 Introduction

Bone is a dynamic tissue; it is constantly being broken down and rebuilt in order to replace old, damaged bone and replace it with new bone tissue to prevent brittleness. This process of bone turnover is carried out by osteoclasts and osteoblasts. Osteoclasts are responsible for breaking down and absorbing nutrients from the old bone, and osteoblasts work to lay down a new bone matrix. When the rate of osteoclast activity is equal to the rate of osteoblast activity, bone mass remains constant. As individuals age, there is a change in the balance of bone turnover; osteoclast activity exceeds osteoblast activity, leading to an overall decrease in bone mass. Bones with low mineral density are weaker, and more susceptible to breaks and fractures (Cummings and Melton, 2002, Goulding et al., 2001).

Osteoporosis is a bone degenerative disorder characterised by low BMD. Osteoporosis is defined as having a BMD t-score of less than -2.5 measured by DXA (*Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group*, 1994). The International Osteoporosis Foundation estimates that osteoporosis affects 1 in 3 women and 1 in 5 men over the age of 50 (Kanis et al., 2000, Melton et al., 1992, Melton et al., 1998). Individuals suffering from osteoporosis can have a resultant decrease in levels of physical activity, causing increased risk factor for weight gain, heart disease, and diabetes. A loss of physical activity ability and independence can also lead to reduced quality of life (Chodzko-Zajko, 2014).

Adult women can undergo a large loss of bone mass following the menopause, as a result of the decrease in oestrogen levels (Khosla et al., 2012, Lanham-New, 2008, Sambrook et al., 1993, Seifert-Klauss et al., 2012). Individuals who have a greater peak bone mass before undergoing the menopause are less at risk for developing osteoporosis. Increasing bone mass and bone formation in comparison to bone resorption before the menopause may therefore result in a decrease in one risk factor for postmenopausal osteoporosis and fracture.

Bone mineralisation is a high-energy process (Freemont, 1993). Creatine supplementation increases the body's stores of PCr (Greenhaff et al., 1994), which in turn allows more resynthesis of ATP, allowing the body to perform more high-energy work through the PCr system. Creatine supplementation can result in increases in fat-

free mass in elite male athletes (Kreider et al., 1998a). Increases in fat-free mass result in increased forces for bone loading, due to increased pull of muscle on bone at sites of attachment. Increased bone loading through increased muscle mass may in turn lead to increases in bone formation, in accordance with Wolff's Law and the Mechanostat theory (Frost, 1994, Schoenau, 2005).

Creatine supplementation has the potential to result in increased osteoblast activity and bone mineralisation (Gerber et al., 2005), and can cause increases in markers of bone growth, and decreases in markers of bone resorption (Louis et al., 2002). Creatine supplementation results in increases in BMD in young boys suffering from muscular dystrophy (Louis et al., 2002, Tarnopolsky et al., 2004b) and in rodent models of osteoporosis (Antolic et al., 2007). Although previous research in this thesis did not show significant effects of long-term, low-dose creatine supplementation on bone, a protective effect against muscle wastage was demonstrated in adult women (Study 4). Given the relationship between muscle and bone, there is therefore a potential for long-term, low-dose creatine supplementation to protect against bone loss, which may have a protective effect on bone, in line with Wolff's Law.

Bone adapts to loading by increasing density and therefore strength along the lines of loading. Bones which routinely undergo loading therefore experience increased bone formation due to compression of the bone stimulating osteoblast differentiation and activity (Kang et al., 2011, Weinbaum et al., 1994). Increased osteoblast activity results in decreased osteoclast activity due to the secretion of OPG (Yasuda et al., 1998). Regular impact on the bone results in fractures in the microarchitecture; as the bone heals, it strengthens along the lines of stress. Exercise can also result in increases in muscle mass, and subsequent increased stress on the bone due to the muscle through the muscle-bone unit (Schoenau, 2005), causing additional increases in the loading of bone at muscle attachment sites, and subsequent increases in bone formation.

Physical activity throughout life is important for the development and maintenance of bone mass (Chilibeck et al., 1995); weight-bearing activity during adolescence has been suggested to be a greater predictor for peak bone mass than calcium intake (Welten et al., 1994). Weight-bearing and high-impact exercise results in increases in BMD at the sites which undergo loading (Etherington et al., 1996, Taaffe et al., 1997). Athletes who take part in impact activities have significantly higher BMD than their sedentary

counterparts (Heinonen et al., 1993), and 8-12 months of weight-bearing exercise results in increases in BMD (Bennell et al., 1997, Snow-Harter et al., 1992). While frequent weight-bearing exercise has been shown to be associated with increased bone mass, power athletes have been shown to have significantly higher bone mass at sites subject to high loading than endurance athletes (Bennell et al., 1997). Athletes who take part in non-impact exercise such as cycling and swimming do not exhibit significantly greater BMD than sedentary counterparts (Duncan et al., 2002, Nichols et al., 2003, Taaffe et al., 1995), suggesting that the effect of impact exercise on bone is not due purely to the effect of increased muscle mass.

The introduction of resistance training results in significant increases in muscle mass and strength in endurance athletes and in sedentary individuals (Charette et al., 1991, Chilibeck et al., 1998). Resistance training results in increases in BMD in adolescent females, and in older men and women, and in premenopausal women (Blimkie et al., 1996, Kerr et al., 2001, Lohman et al., 1995a, Maddalozzo and Snow, 2000, Nichols et al., 2001), although findings suggest that increases in BMD of the lumbar spine requires higher intensity exercise than the hip; adaptations to exercise therefore appear to be site-specific (Blimkie et al., 1996, McCartney et al., 1995, Nichols et al., 2001, Pruitt et al., 1995). Resistance exercise, and the combination of resistance and high impact exercise, have been shown to be effective at improving measures of bone health in premenopausal and postmenopausal women (Kelley et al., 2001, Nikander et al., 2010b, Wallace and Cumming, 2000, Wolff et al., 1999).

Half of the strength gains which are achieved through 1 year of training are achieved during the first 12 weeks of a training program in older adults (Latham et al., 2004). A 48 hour rest in between training bouts is generally recommended to allow muscle recovery and development (Bickel et al., 2005, Garber et al., 2011, Pollock et al., 1998), meaning that training 3 days per week is common. Training 2 days per week results in up to 90% of the training adaptations achieved through training 3 days per week in untrained individuals (Demichele et al., 1997); this gives more time for recovery in between training bouts, and is less time consuming, therefore increasing adherence to the exercise program.

The effects of creatine supplementation on body composition and bone may be augmented by the combination of supplementation with a training routine. The effect of loading on the bone from exercise may be synergistic to the effects of creatine on bone metabolism, and the inclusion of an exercise program may increase the body's uptake and utilisation of ingested creatine. Creatine supplementation has been shown to result in increases in BMD in boys suffering from muscular dystrophy who are able to walk, and not in those who are chair-bound, suggesting that there is a combined effect of creatine and physical activity on bone health (Louis et al., 2002). Resistance training in combination with creatine supplementation results in preservation of femoral BMD in postmenopausal women and in older men, and results in decreases in bone resorption (Candow et al., 2008, Chilibeck et al., 2005, Chilibeck et al., 2014). Creatine supplementation in combination with resistance training has been suggested to have potential to be an effective strategy for minimising loss of bone mass (Candow and Chilibeck, 2010).

The present study therefore aims to investigate the effects of creatine monohydrate supplementation in combination with combined resistance and high impact exercise on BMD in adult women. The secondary aim is to investigate the effects of creatine monohydrate supplementation on markers of bone turnover in adult women. The present study also aims to investigate the effects of creatine monohydrate supplementation in combination with resistance exercise on functional muscle strength and fitness measures. It was hypothesised that creatine supplement alone, and exercise alone, will have a beneficial effect on BMD, bone turnover, and functional strength, and that creatine in combination with exercise will have a synergistic effect on BMD, bone turnover, and functional strength.

8.3 Methods

8.3.1 Participants

Forty-five female participants (Age = 42.4 ± 9.2 years; Height = 1.66 ± 0.06 m; Mass = 72.8 ± 13.9 kg; BMI = 26.4 ± 4.8 kg·m⁻²) volunteered to take part in this study. Participants were randomly assigned in a double-blind manner to one of four groups; exercise plus creatine, exercise with placebo, non-exercise with creatine, and non-exercise with placebo. Randomisation was done using block randomisation with block size 8 to maintain equal group sizes across conditions. Throughout the study, seven

participants withdrew; three due to family commitments, two due to work commitments, and two due to injury not associated with the intervention. Thirty-eight participants (Age = 44.2 ± 9.5 years, Height = 1.66 ± 0.06 m, Mass = 73.3 ± 14.0 kg, BMI = 26.6 ± 5.0 kg·m⁻²) completed the study, with no significant differences at baseline in age ($F=1.692$, $p=0.187$), height ($F=0.220$, $p=0.882$), mass ($F=0.510$, $p=0.678$), or BMI ($F=0.492$, $p=0.690$). Participant characteristics of each group are shown in Table 8.1.

Table 8.1: Participant Characteristics. All values shown are mean \pm standard deviation. BMI = Body Mass Index.

		n	Age (years)	Height (m)	Mass (kg)	BMI (kg·m ⁻²)
Creatine	Exercise	10	41.8 ± 7.1	1.66 ± 5.8	72.5 ± 12.0	26.2 ± 3.5
	Control	10	47.2 ± 10.1	1.66 ± 7.9	69.3 ± 12.0	25.2 ± 3.6
Placebo	Exercise	9	39.9 ± 11.7	1.66 ± 4.5	76.0 ± 17.0	27.9 ± 7.2
	Control	9	47.9 ± 7.6	1.68 ± 6.2	76.2 ± 16.1	27.1 ± 5.4

8.3.2 Experimental Design

The study was a double-blind randomised controlled trial. Participants made two visits to the laboratory following a 4-hour fast, pre- and post-intervention. During each visit all participants underwent DXA scans (proximal femur, lumbar spine, and whole-body), strength and fitness tests, provided a venous blood sample, and completed a 3-day food diary, and the long iPAQ (Craig, 2003) (see Appendix N. iPAQ (long). Full details of all procedures are given in Chapter 3: General Methods.

8.3.3 Procedures

8.3.3.1 Pre- and post-testing

All measures were completed at baseline and were repeated at the end of the intervention period for all participants. For each visit where measures were taken, participants attended the laboratory following a 4-hour fast, with a controlled fluid

intake of 500ml during the fasting period. Participants were requested to refrain from any form of physical activity for 24 hours prior to testing.

8.3.3.2 *Anthropometric Measurements*

Participants' height was measured with a stadiometer without shoes and the minimum clothes to the nearest 0.1 cm (Harpendon Stadiometer [Holtain Ltd, Crymych, UK]). A scale with a 200 kg maximum capacity and 0.1 kg precision (Seca 899, Seca gmb & co, Hamburg, Germany) was used to measure participants' weight.

8.3.3.3 *DXA Scans*

Participants lay on the bed in the supine position, and hip and spine positioning devices were used for the BMD scans. For the whole body scan, participants were asked to lie still in the supine position with their arms by their sides. Bone mineral density was obtained for each participant by DXA scan (Hologic Discovery QDR A; APEX System Software Version 3.4.2) in supine position, of the proximal femur, lumbar spine and whole body (for a more detailed procedure, see 3.3.3).

8.3.3.4 *Blood sample collection*

Serum blood samples were obtained for each participant from the medial antecubital vein. Samples were left at room temperature ($20^{\circ}\text{C} \pm 5^{\circ}\text{C}$) for between 75 and 90 minutes, and then centrifuged at $1300 \times g$ for 11 minutes at 4°C . Serum from samples was then removed and stored at -80°C for later analysis. Full details of the blood sampling procedure are given in 3.5.1.

8.3.3.5 *Blood Analysis*

Serum samples were analysed for biochemical markers of bone turnover; Osteocalcin (Olc) and N-terminal teleopeptide (NTX) using the ELISA technique, and Alkaline Phosphatase (ALP) using the Randox RX Daytona Plus (Randox Laboratories Ltd., County Antrim, UK) through direct photometry. Serum creatinine was also measured using the Randox RX Daytona Plus (Randox Laboratories Ltd., County Antrim, UK). Full details of the techniques used can be found in 3.5.2.

8.3.3.6 *Isokinetic Leg Strength*

Participants completed isokinetic strength tests on an isokinetic dynamometer (Biodex [Biodex Isokinetic System III, IPRS Mediquipe, Little Blakenham, UK]) to measure functional strength of the knee extensors and flexors of the left leg. Participants were seated on the Biodex, and performed 5 maximal extensions and contractions at 2 angular velocities; $60^{\circ}\cdot\text{sec}^{-1}$ and $120^{\circ}\cdot\text{sec}^{-1}$. Full details of the procedure are given in 3.4.

8.3.3.7 *Fitness Measures*

Participants underwent a series of fitness measures designed to establish functional fitness; standard fitness measures designed to assess the functionality of various different muscle groups were used. These measures consisted of stand-and-sit, jump height, handgrip strength, back strength, 1-leg balance, 50m walk, and sit-and-reach.

8.3.3.7.1 *Stand and sit*

Participants were seated on a chair, and were instructed to stand up and sit down as many times as possible in 30 seconds, timed using a stopwatch. Standing up involved standing until the knees were straight, and sitting down included touching the seat of the chair. The investigator counted the number of complete repetitions performed in the 30 second period.

8.3.3.7.2 *Jump height*

Participants strapped a jump height dynamometer around their waist, and stood on the attached rubber mat. Participants were instructed to jump as high as they could; the test was repeated three times, and the maximum jump height was recorded.

8.3.3.7.3 *Handgrip strength*

Participants held a handgrip dynamometer in their dominant hand, and stood holding the dominant arm straight out from the shoulder, parallel to the floor. Participants were instructed to squeeze the dynamometer using as much force as possible, while bringing the dominant arm slowly down to their side. Verbal encouragement was given

throughout. The test was repeated three times, and the highest of the three values was recorded.

8.3.3.7.4 Back strength

Participants held the handle of a back strength dynamometer, while standing securely on the base. The length of the dynamometer chain was manually adjusted to fit each participant, so that the chain was at full length when participants stood with knees slightly bent and the back straight and upright. Participants then pulled upwards with as much force as possible, while keeping the back straight. The test was repeated three times, and the highest of the three values was recorded. If any discomfort was experienced, the test was stopped immediately.

8.3.3.7.5 1-leg balance

Participants stood on level flooring, and lifted one leg off the ground, resting the lifted foot on the ankle of the foot remaining on the floor. A stopwatch was used to time how long the position could be maintained, for a maximum of 60 seconds. Participants were given the option of repeating the test on the other leg; the leg used was recorded.

8.3.3.7.6 50m walk

Two lines were drawn on the floor, 5m apart. Participants stood behind one line, and were instructed to walk to the other line and back again, five times, touching each line each time. The total distance walked was 50m; the stopwatch was stopped as the participant crossed the line for the final time.

8.3.3.7.7 Sit and reach

Participants sat on the floor, with both legs outstretched in front of them. The sit-and-reach box was placed touching the feet, and participants were instructed to reach forwards as far as possible towards their feet. The value reached on the box with outstretched fingers was recorded.

8.3.4 Training Program

The exercise group undertook 24 exercise sessions over 12 weeks. Participants attended 2 exercise sessions each week, with a gap of at least 48 hours between each session.

Before each session, participants underwent a 5-minute warm-up on a stationary cycle ergometer (Monark, 874E, Varberg, Sweden), with minimal resistance. Each session then consisted of 12 stations, alternating between aerobic and resistance exercises. Participants completed 60 seconds at each station, with a 30 second break in between each station. The exercises used included; bicep curls, press-ups, sit-ups, jumping jacks, skipping, stationary cycling, stationary running, and step-ups. Throughout each exercise session, all major muscle groups were utilised. For full details of the exercises performed, see Appendix O. Exercises used in sessions Participants progressed in exercise volume when they were able to comfortably complete 10 repetitions of an exercise. The total time commitment for each session was approximately 60 minutes, including a warm up and warm down. All participants assigned to the exercise program completed a minimum of 80% of the exercise sessions (20/24).

All participants, regardless of group, were instructed to maintain habitual levels of physical activity. This was assessed using the long IPAQ, pre- and post-intervention. No significant changes were recorded in habitual physical activity levels in any group across the period of the intervention.

8.3.5 Supplementation

Participants were instructed to take 3g per day of either Creatine Monohydrate (CreaPure, AlzChem, Trostberg, Germany), or of a taste- and appearance-matched placebo (micro-crystalline cellulose, Blackburn Distribution Ltd., Lancashire, UK). Participants were instructed to mix the supplement powder with water, and to consume with a meal, preferably with breakfast. Participants were initially given 2 weeks' worth of supplement (42g); this was renewed fortnightly throughout the study, and the total amount given to each participant was recorded. Any supplement remaining at the end of the study was returned to the laboratory and re-weighed. All participants achieved a minimum of 80% adherence to the supplementation protocol (202/252g).

8.3.6 Statistical Analysis

All data were analysed using SPSS for Windows (SPSS for Windows, Version 21.0, SPSS Inc., Chicago). Data were initially analysed for normality. Participant characteristics were compared across groups using 1-way ANOVA. Mixed between-within ANOVA was used to establish whether there was interaction between Creatine

supplement and exercise for; femur neck BMD, proximal femur BMD, lumbar spine BMD, Olc, ALP, NTX, fat-free mass, fat mass, fat percentage, and leg strength. Data are presented as mean \pm standard deviation, with alpha level set at 0.05.

8.4 Results

One-way ANOVA revealed that there were no significant differences in participant characteristics across the 4 participant groups at baseline. Participant characteristics are summarised in Table 8.1. BMD and blood biomarker data pre- and post-intervention are presented in Table 8.2.

Mixed between-within subjects analysis of variance was conducted to assess the impact of supplementation and exercise on BMD, blood biomarkers, body composition, and strength and fitness measures. The within-subjects factor was time, and between-subjects factors were supplement and exercise. All data met the assumptions of homogeneity of variance and homogeneity of intercorrelations (Levene's Test $p > 0.05$, Box's Test $p > 0.01$).

Table 8.2: BMD and biomarker values pre- and post-intervention across 4 groups. Creatine/Exer = Creatine supplement with exercise, Control/Exer = Control supplement with exercise, Creatine/NonExer = Creatine supplement without exercise, Control/NonExer = Control supplement without exercise. All data are presented as mean \pm standard deviation. * denotes statistically significant difference from baseline ($p < 0.05$).

	Creatine/Exer	Control/Exer	Creatine/NonExer	Control/NonExer
Baseline				
Femur Neck BMD (g.cm ⁻²)	0.87 \pm 0.15	0.87 \pm 0.12	0.75 \pm 0.08	0.85 \pm 0.12
Total Hip BMD (g.cm ⁻²)	1.01 \pm 0.14	0.99 \pm 0.13	0.90 \pm 0.08	0.98 \pm 0.11
Spine BMD (g.cm ⁻²)	1.05 \pm 0.11	0.94 \pm 0.09	0.99 \pm 0.13	0.98 \pm 0.12
Post-Intervention				
Femur Neck BMD (g.cm ⁻²)	0.85 \pm 0.14	0.86 \pm 0.11	0.75 \pm 0.08	0.84 \pm 0.12
Total Hip BMD (g.cm ⁻²)	1.00 \pm 0.14	0.98 \pm 0.13	0.90 \pm 0.08	0.98 \pm 0.11
Spine BMD (g.cm ⁻²)	1.05 \pm 0.11	0.95 \pm 0.10	0.99 \pm 0.16	1.00 \pm 0.1
Baseline				
NTX (ng.ml ⁻¹)	86.20 \pm 55.87	212.12 \pm 127.86	132.77 \pm 88.66	110.27 \pm 100.80
Olc (pmol.L ⁻¹)	12.57 \pm 9.30	7.14 \pm 3.85	13.92 \pm 4.89	14.45 \pm 8.10
ALP (μ .L ⁻¹)	49.64 \pm 19.35	63.13 \pm 24.18	52.13 \pm 21.38	62.81 \pm 11.50
Creatinine (μ mol.L ⁻¹)	66.11 \pm 9.44	72.90 \pm 8.66	71.49 \pm 9.72	70.21 \pm 10.79
Post-Intervention				
NTX (ng.ml ⁻¹)	82.86 \pm 42.34	121.79 \pm 86.38*	135.04 \pm 105.38	99.21 \pm 55.19
Olc (pmol.L ⁻¹)	15.74 \pm 11.42*	12.20 \pm 7.70	16.75 \pm 7.61	14.07 \pm 8.08
ALP (μ .L ⁻¹)	43.73 \pm 20.39	46.92 \pm 19.94*	41.69 \pm 13.38	52.52 \pm 12.50
Creatinine (μ mol.L ⁻¹)	62.37 \pm 8.81	56.29 \pm 12.32	65.30 \pm 11.64	60.04 \pm 10.00

8.4.1 Femur Neck BMD

Mixed between-within ANOVA revealed that there was a significant interaction between exercise and time ($F(1,33)=4.70$; $p=0.04$; partial eta squared=0.125), with the exercise participants exhibiting a greater decrease in femur neck BMD than control. The observed decrease did not exceed the LSC calculated in Study 1 (see 4.4). There was a significant main effect of time on femur neck BMD ($F(1,33)=6.06$; $p=0.02$; partial eta squared=0.155), with all groups showing a decrease in femur neck BMD across time; see Figure 8.1. The main effect comparing supplement protocol was not significant ($F(3,33)=1.40$; $p=0.25$; partial eta squared=0.041). The main effect comparing exercise protocol was not significant ($F(3,33)=2.86$; $p=0.10$; partial eta squared=0.080). The main effect comparing the interaction of supplement and exercise was not significant ($F(3,33)=1.31$; $p=0.26$; partial eta squared=0.038).

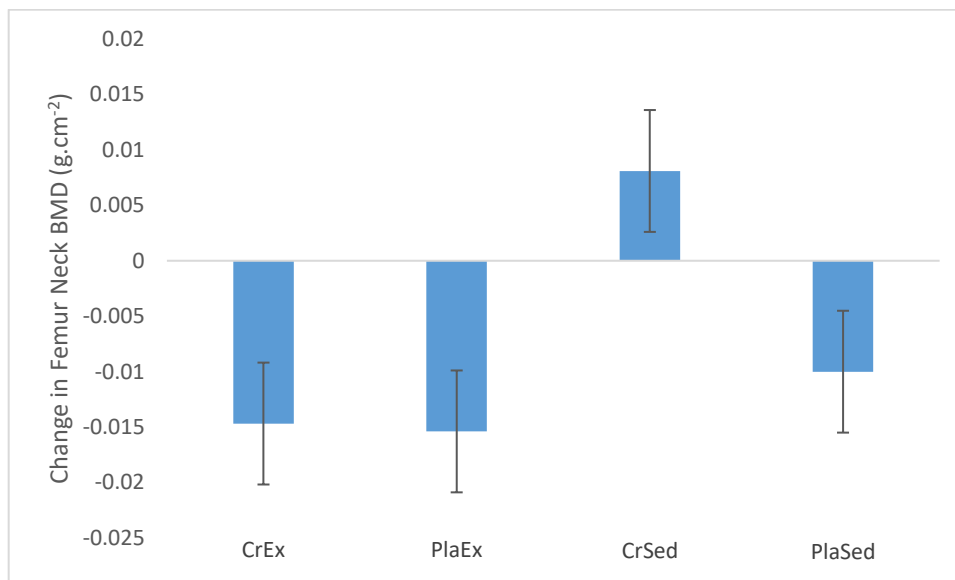


Figure 8.1: Change in BMD of the femur neck across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.2 Proximal Femur BMD

Mixed between-within ANOVA revealed that there was a significant effect of time on femur BMD ($F(1,33)=7.28$; $p=0.01$; partial eta squared=0.181), with a general decrease in proximal femur BMD across time; see Figure 8.2. There was no significant interaction between group and time ($F(1,33)=0.03$; $p=0.87$; partial eta squared=0.001).

The main effect of supplement was not significant ($F(3,33)=0.55$; $p=0.47$; partial eta squared=0.016). The main effect comparing the interaction of supplement and exercise was not significant ($F(3,33)=1.65$; $p=0.21$; partial eta squared=0.048).

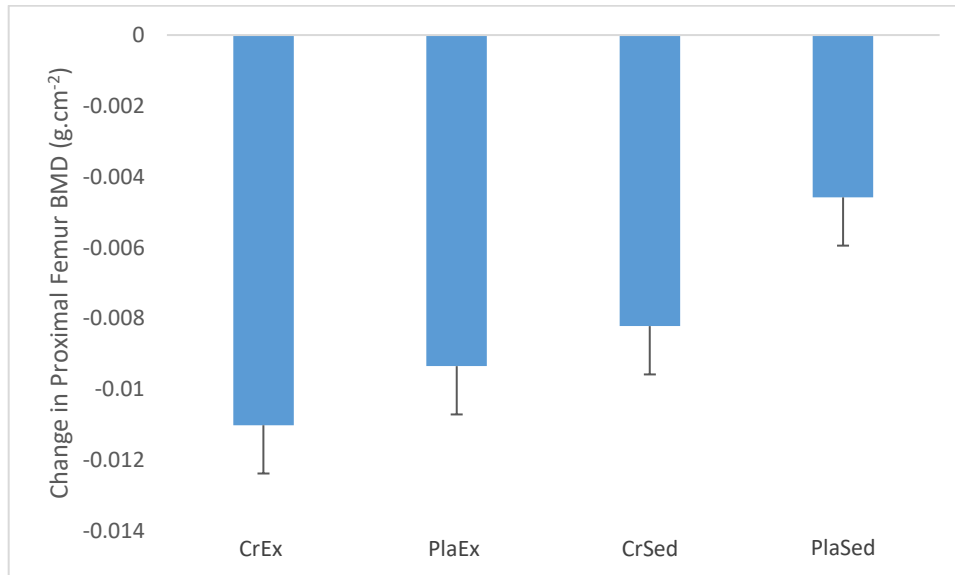


Figure 8.2: Change in BMD of the proximal femur across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.3 Lumbar Spine BMD

Mixed between-within ANOVA revealed that there was no significant interaction of time and group on lumbar spine BMD ($F(1,34)=0.74$; $p=0.40$; partial eta squared=0.021). The main effect of supplement on lumbar spine BMD was not significant ($F(3,34)=1.78$; $p=0.19$; partial eta squared=0.050). There was no significant effect of exercise ($F(3,34)=0.05$; $p=0.82$; partial eta squared=0.001). There was no significant interaction between supplement and exercise on lumbar spine BMD ($F(3,34)=1.72$; $p=0.20$; partial eta squared=0.048).

8.4.4 Osteocalcin

Mixed between-within ANOVA revealed that there was a substantial main effect for time ($F(1,34)=7.74$; $p=0.01$; partial eta squared=0.185), with a general increase in Olc level through the intervention; only the placebo non-exercise group showed a decrease in Olc across time; see Figure 8.3. There was no significant main effect of creatine

supplement on Olc ($F(3,34)=1.33$; $p=0.26$; partial eta squared=0.038). There was no significant effect of exercise on Olc ($F(3,34)=1.44$; $p=0.24$; partial eta squared=0.041). There was no significant interaction between supplementation/exercise and time ($F(3,34)=0.50$; $p=0.48$; partial eta squared=0.015).

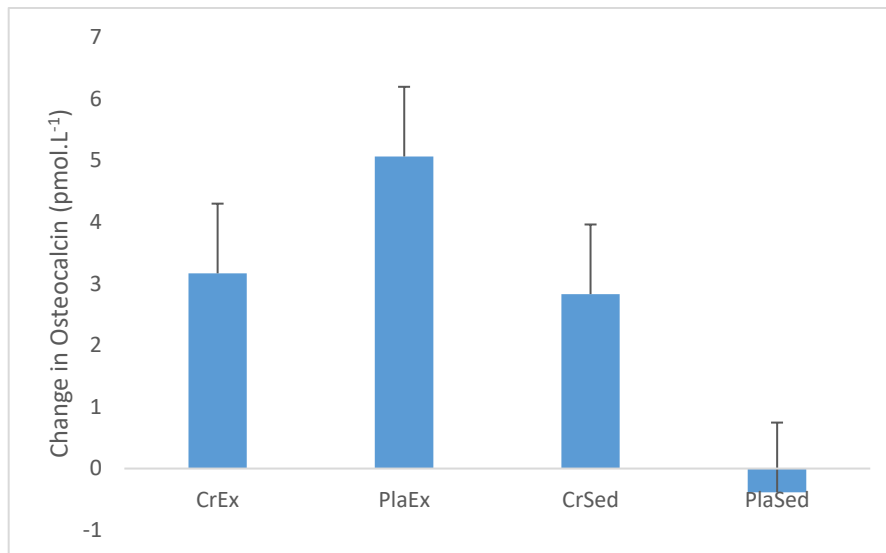


Figure 8.3: Change in Olc levels across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.5 Alkaline Phosphatase

Mixed between-within ANOVA revealed that there was a substantial main effect for time ($F(1,34)=23.83$; $p<0.001$; partial eta squared=0.412), with an overall decrease in ALP level through the intervention; see Figure 8.4. There was no significant main effect of supplement on ALP ($F(3,34)=2.95$; $p=0.10$; partial eta squared=0.080). There was no significant main effect of exercise ($F(3,34)=0.07$; $p=0.80$; partial eta squared=0.002). There was no significant interaction between supplement and exercise ($F(3,34)=0.05$; $p=0.83$; partial eta squared=0.001).

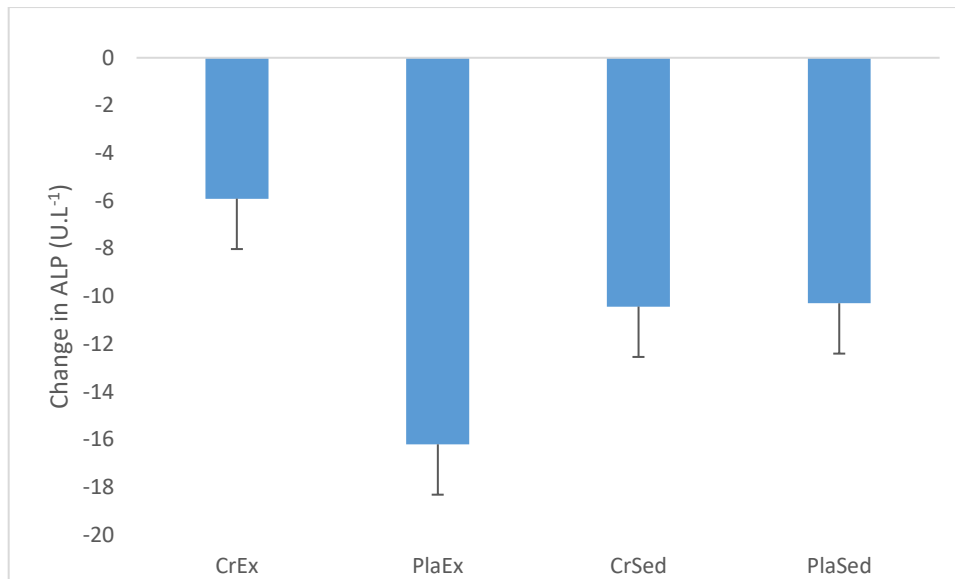


Figure 8.4: Change in ALP levels across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.6 N-Telopeptides

Mixed between-within ANVOA revealed that there was a significant main effect of time ($F(1,34)=6.15$; $p=0.02$; partial eta squared=0.153); see Figure 8.5. There was a significant interaction between supplement and time ($F(1,34)=5.90$; $p=0.02$; partial eta squared=0.148), with the control group exhibiting a greater decrease in NTX across the intervention. There was a significant interaction of exercise and time ($F(1,34)=4.23$; $p=0.05$; partial eta squared=0.111), with the exercise group exhibiting a greater decrease in NTX across the intervention than control. The main effect of supplement was not significant ($F(3,34)=1.03$; $p=0.32$; partial eta squared=0.029). The main effect of exercise was not significant ($F(3,34)=0.06$; $p=0.81$; partial eta squared=0.002). There was a significant interaction between supplement and exercise on NTX ($F(3,34)=4.53$; $p=0.04$; partial eta squared=0.118), with the control supplement plus exercise group exhibiting a greater decrease in NTX levels.

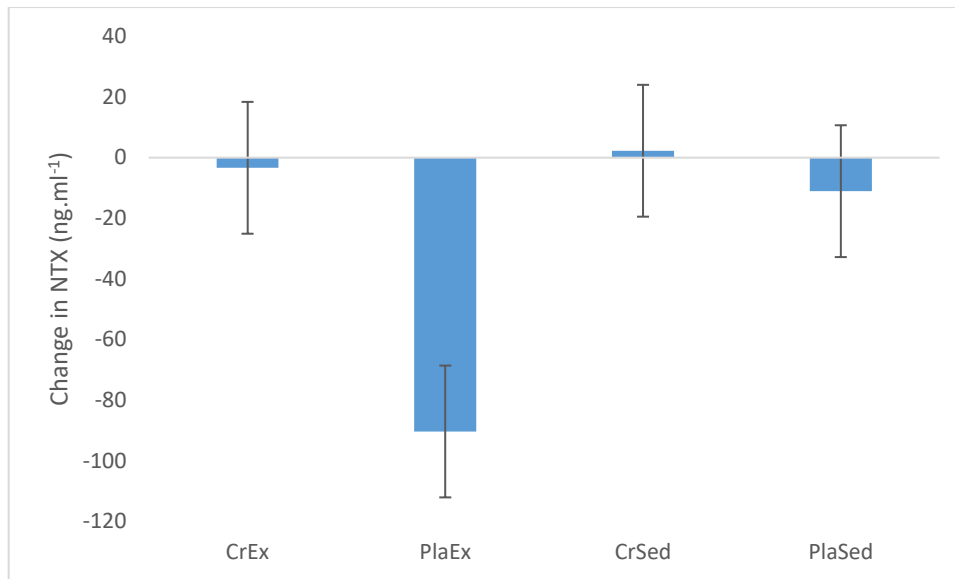


Figure 8.5: Change in NTX across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.7 Bone Turnover Ratio

For purposes of statistical analysis, the NTX:ALP ratio was calculated as NTX/ALP . Mixed between-within ANOVA revealed that there was no significant interaction of time and group on NTX:ALP ratio ($F(1,33)=0.49$; $p=0.49$; partial eta squared=0.015). There was no significant main effect of creatine supplementation on NTX:ALP ratio ($F(3,34)=0.00$; $p=0.99$; partial eta squared=0.000). There was no significant main effect of exercise on NTX:ALP ratio ($F(3,34)=0.257$; $p=0.62$; partial eta squared=0.008). There was no significant interaction of supplement and exercise on NTX:ALP ratio ($F(3,34)=3.82$; $p=0.06$; partial eta squared=0.101).

8.4.8 Fat-free Mass

Mixed between-within ANOVA revealed that there was a significant interaction of exercise and time on upper limb fat-free mass ($F(1,34)=5.40$; $p=0.03$; partial eta squared=0.137), with exercise resulting in increased upper limb fat-free mass in comparison to control; see Figure 8.6. This change exceeded the LSC calculated in Study 1 (see 4.4). There was no significant main effect of supplement ($F(3,34)=0.21$; $p=0.65$; partial eta squared=0.006) or exercise ($F(3,34)=0.06$; $p=0.82$; partial eta

squared=0.002). There was no significant main effect of supplement and exercise on upper limb fat-free mass ($F(3,34)=0.13$; $p=0.72$; partial eta squared=0.004).

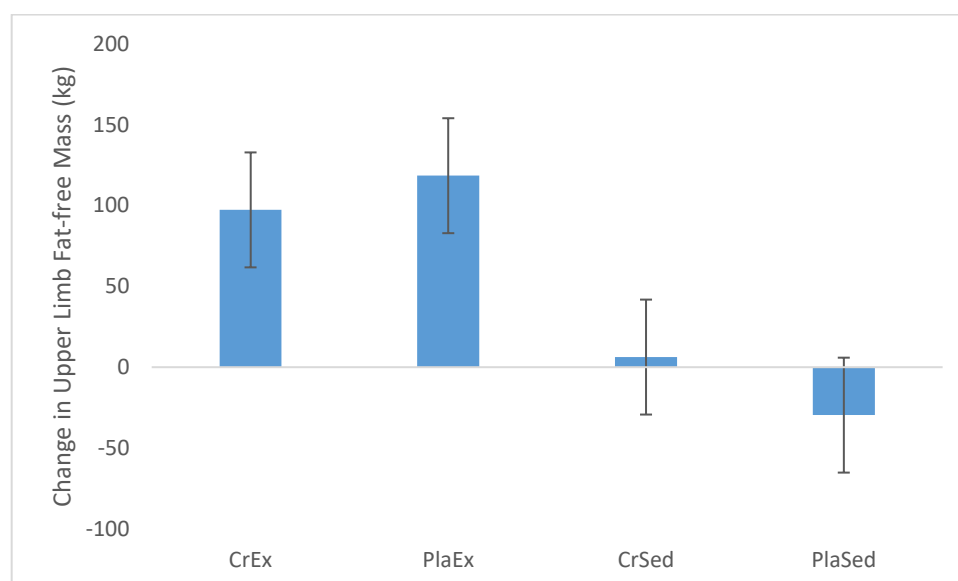


Figure 8.6: Change in upper limb fat-free mass across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

There was a significant main effect of time on lower limb fat free mass ($F(1,34)=4.22$; $p=0.05$; partial eta squared=0.11), with an overall decrease in lower limb fat free mass across the intervention; see Figure 8.7. There was no significant interaction between time and group on lower limb fat-free mass ($F(1,34)=0.85$; $p=0.36$; partial eta squared=0.024) or subtotal fat-free mass ($F(1,34)=1.08$; $p=0.31$; partial eta squared=0.031). There was no significant main effect of supplement on fat-free mass of the lower limbs ($F(3,34)=1.89$; $p=0.18$; partial eta squared=0.053) or subtotal whole body ($F(3,34)=1.21$; $p=0.28$; partial eta squared=0.034). There was no significant main effect of exercise on fat-free mass of the lower limbs ($F(3,34)=0.59$; $p=0.45$; partial eta squared=0.017) or subtotal whole body ($F(3,34)=0.12$; $p=0.73$; partial eta squared=0.004). There was no significant interaction of supplement and exercise on fat-free mass of the lower limbs ($F(3,34)=0.34$; $p=0.56$; partial eta squared=0.010) or subtotal whole body ($F(3,34)=0.36$; $p=0.56$; partial eta squared=0.010).

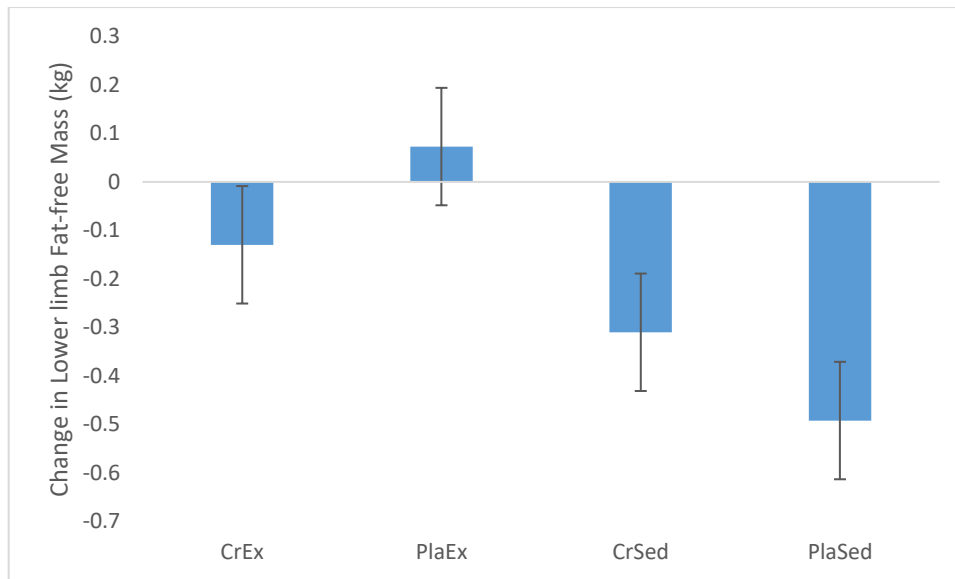


Figure 8.7: Lower limb fat-free mass across groups, pre- and post-intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.9 Fat Mass

Mixed between-within ANOVA revealed that there was a significant interaction between time and exercise on fat mass of the upper limbs ($F(1,34)=6.09$, $p=0.02$, partial eta squared=0.152), of the lower limbs ($F(1,34)=5.36$, $p=0.03$, partial eta squared=0.14), and of the subtotal whole body ($F(1,34)=4.50$, $p=0.04$, partial eta squared=0.117), with a decrease in fat mass following the exercise program, and an increase in fat mass in the control group; see Figure 8.8, Figure 8.9, Figure 8.10. This change did not exceed the LSC calculated in Study 1 (see 4.4) There was no significant main effect of supplement on fat mass of the upper limbs ($F(3,34)=0.65$, $p=0.43$, partial eta squared=0.019), of the lower limbs ($F(3,34)=1.08$, $p=0.31$, partial eta squared=0.031), or of the subtotal whole body ($F(3,34)=1.53$, $p=0.22$, partial eta squared=0.043). There was no significant main effect of exercise on fat mass of the upper limbs ($F(3,34)=0.12$, $p=0.73$, partial eta squared=0.004), of the lower limbs ($F(3,34)=0.02$, $p=0.88$, partial eta squared=0.001), or of the subtotal whole body ($F(3,34)=0.01$, $p=0.91$, partial eta squared=0.000). There was no significant interaction of supplement and exercise on fat mass of the upper limbs ($F(3,34)=0.61$, $p=0.44$, partial eta squared=0.018), of the lower limbs ($F(3,34)=0.80$, $p=0.38$, partial eta squared=0.018), or of the subtotal whole body ($F(3,34)=0.80$, $p=0.38$, partial eta squared=0.018).

squared=0.023), or of the subtotal whole body ($F(3,34)=0.16$, $p=0.69$, partial eta squared=0.005).

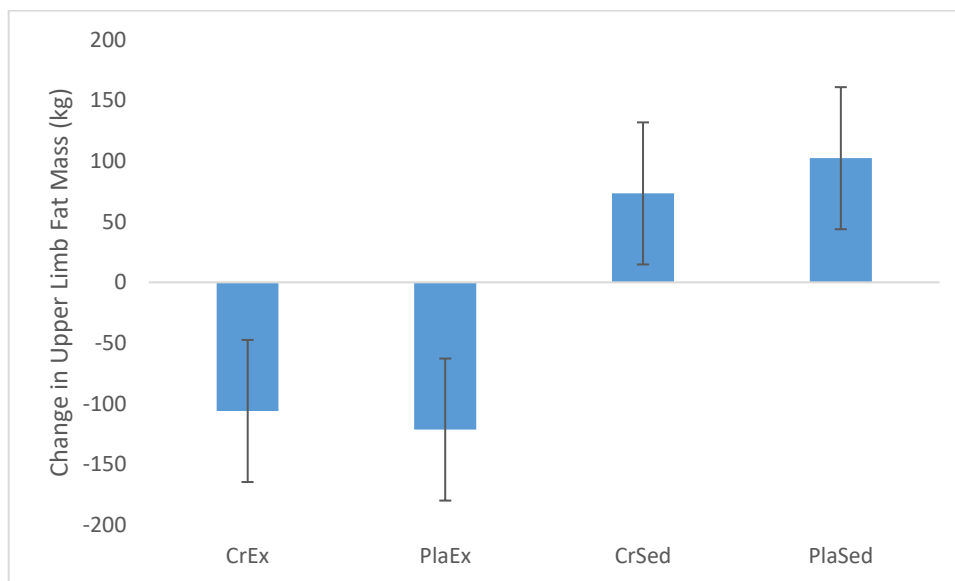


Figure 8.8: Change in upper limb fat mass across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

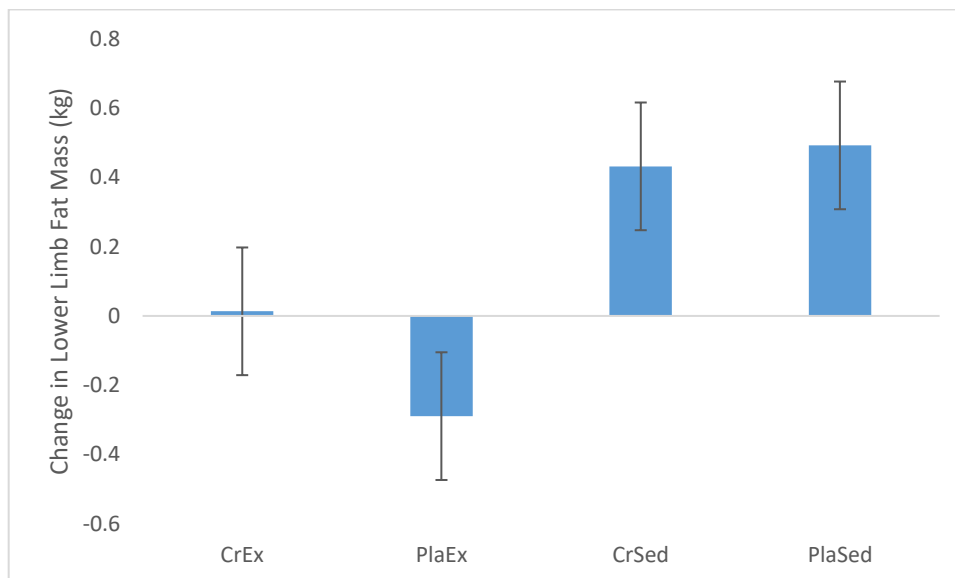


Figure 8.9: Change in lower limb fat mass across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

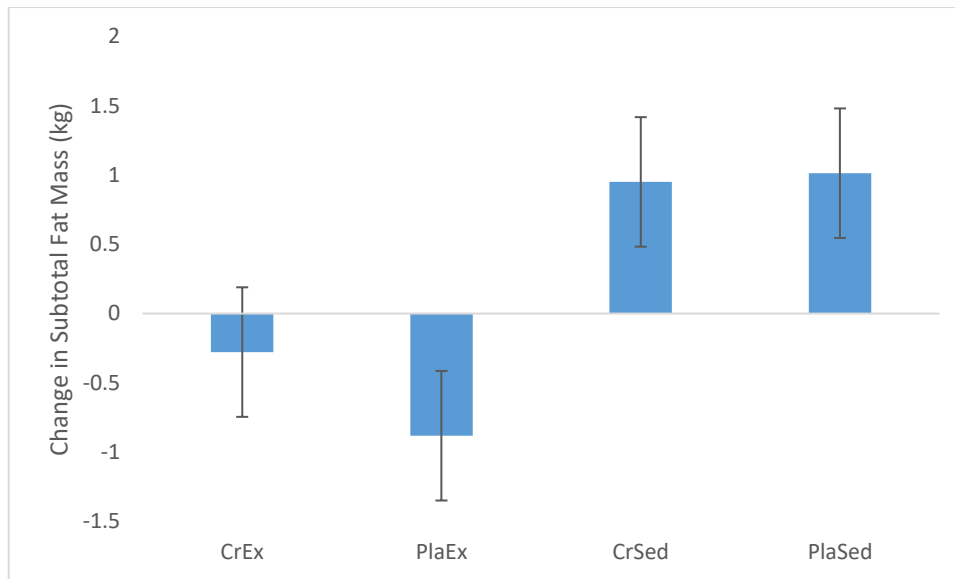


Figure 8.10: Change in subtotal fat mass across the intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.10 Isokinetic Leg Strength

Mixed between-within ANOVA revealed that there was a significant interaction of time and supplement on torque at 60 deg·sec⁻¹ ($F(1,30)=6.22$; $p=0.02$; partial eta squared=0.172), and at 120 deg·sec⁻¹ ($F(1,30)=4.11$; $p=0.05$; partial eta squared=0.121), with participants in the supplement group exhibiting a greater increase in strength in comparison to control; see Figure 8.11, Figure 8.12. There was no significant main effect of supplement on isokinetic strength at 60 deg·sec⁻¹ ($F(3,30)=0.74$; $p=0.40$; partial eta squared=0.024) or at 120 deg·sec⁻¹ ($F(3,30)=1.00$; $p=0.33$; partial eta squared=0.032). There was no significant main effect of exercise on isokinetic strength at 60 deg·sec⁻¹ ($F(3,30)=0.06$; $p=0.81$; partial eta squared=0.002) or at 120 deg·sec⁻¹ ($F(3,30)=0.39$; $p=0.54$; partial eta squared=0.013). There was no significant interaction between supplement and exercise on torque at 60 deg·sec⁻¹ ($F(3,30)=0.09$; $p=0.76$; partial eta squared=0.003), or at 120 deg·sec⁻¹ ($F(3,30)=0.25$; $p=0.62$; partial eta squared=0.008).

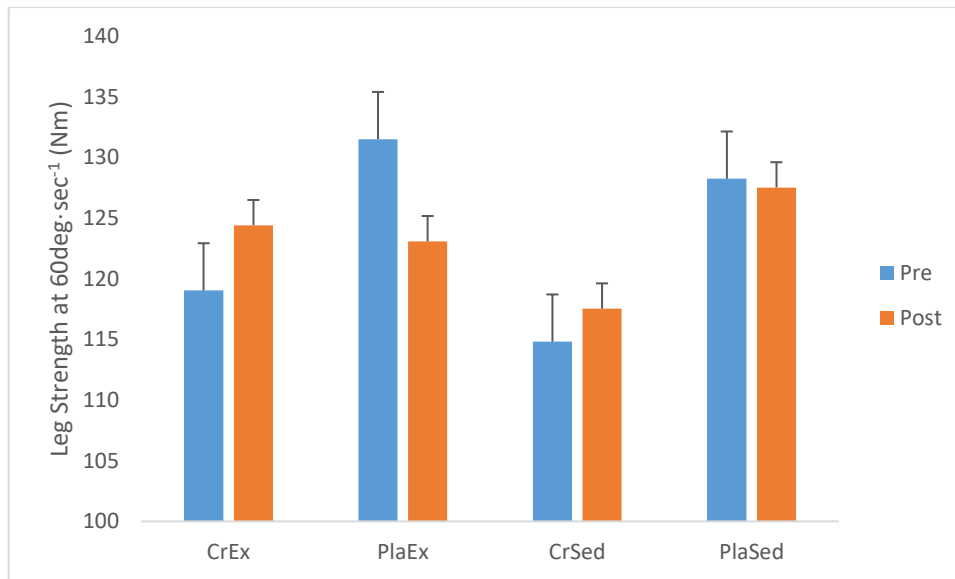


Figure 8.11: Maximal isokinetic torque of the left leg at 60 deg·sec⁻¹, across groups. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

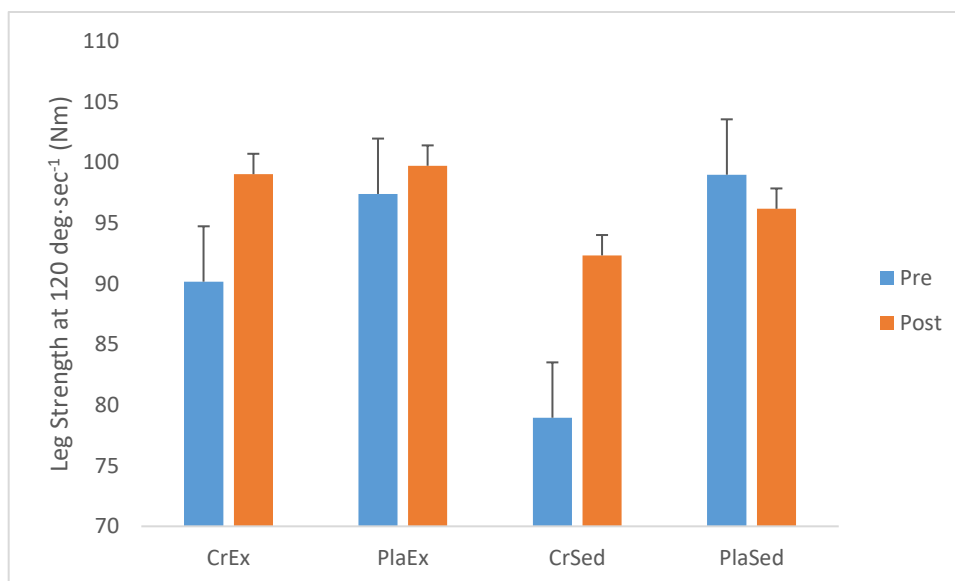


Figure 8.12: Maximal isokinetic torque of the left leg at 120 deg·sec⁻¹, across groups. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.11 Back Strength

Mixed between-within ANOVA revealed that there was no significant main effect of supplement on back strength ($F(3,33)=2.20$; $p=0.15$; partial eta squared=0.062). There

was no significant main effect of exercise on back strength ($F(3,33)=3.77$; $p=0.06$; partial eta squared=0.103). There was no significant interaction between supplement and exercise on leg strength ($F(3,33)=0.83$; $p=0.37$; partial eta squared=0.024).

After adjusting for baseline back strength, between-groups ANCOVA revealed that there was no significant interaction effect of supplement and exercise ($F=0.217$; $p>0.05$; partial eta squared=0.007). There was no statistically significant main effect of supplement ($F=0.198$; $p>0.05$; partial eta squared=0.006). There was a statistically significant main effect of exercise ($F=4.338$; $p=0.045$; partial eta squared=0.119), with the exercise program resulting in increased back strength in comparison to the non-exercise group. There was a strong relationship between the pre-intervention and post-intervention back strength, as indicated by a partial eta squared valued of 0.454; see Figure 8.13.

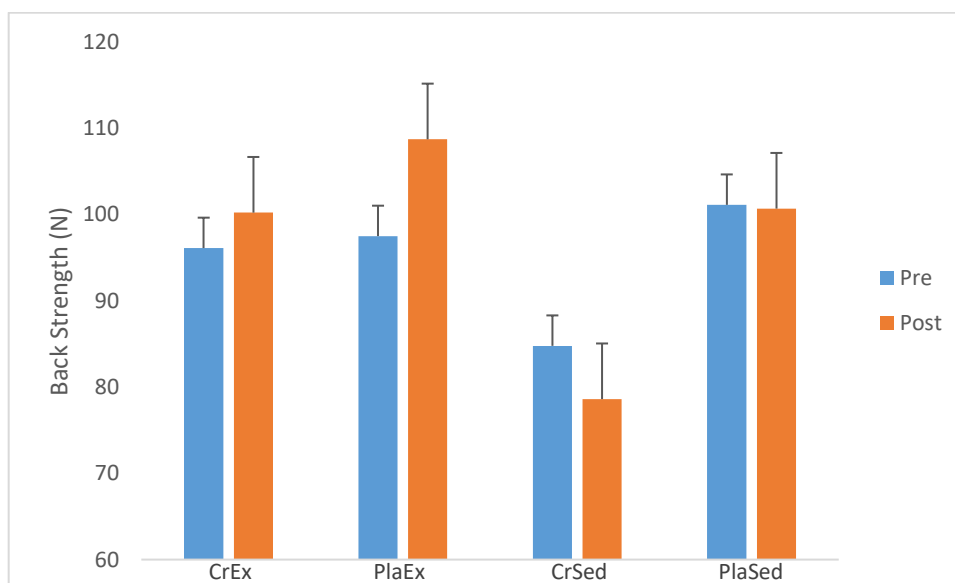


Figure 8.13: Maximal strength of the back extensor muscles across groups, pre- and post-intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.12 Stand and Sit

Mixed between-within ANOVA revealed that there was no significant interaction of time and group on stand-and-sit ($F(1,33)=0.00$; $p=0.97$; partial eta squared=0.000). The main effect of supplement on stand-and-sit performance was not significant

($F(3,33)=1.72$; $p=0.20$; partial eta squared=0.050). There was a significant main effect of exercise on stand-and-sit ($F(3,33)=4.62$; $p=0.04$; partial eta squared=0.123), with the exercise regime resulting in improved stand-and-sit performance; see Figure 8.14. There was no significant interaction between supplement and exercise on stand and sit ($F(3,33)=0.47$; $p=0.50$; partial eta squared=0.014).

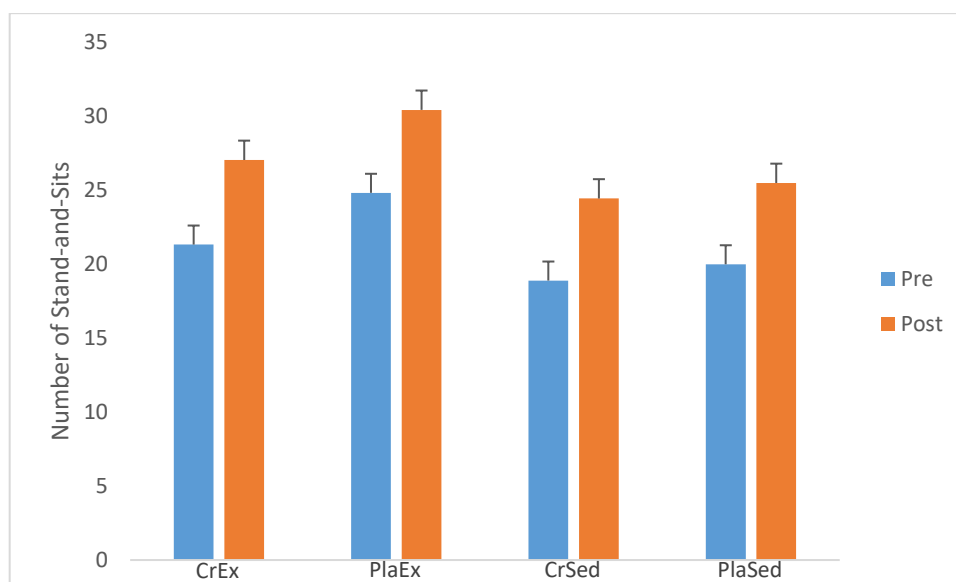


Figure 8.14: Stand and sit performance across groups, pre- and post-intervention; number of stand-and-sits performed in 30 seconds. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.13 50m Walk

Mixed between-within ANVOA revealed that there was no significant interaction of time and group on 50m walk time ($F(1,33)=0.37$; $p=0.55$; partial eta squared=0.011); see Figure 8.15. The main effect of supplement on stand and sit was not significant ($F(3,33)=0.14$; $p=0.71$; partial eta squared=0.004). There was a significant main effect of exercise on 50m walk time ($F(3,33)=5.50$; $p=0.03$; partial eta squared=0.143), with a decreased walk time in the exercise group. There was no significant interaction between supplement and exercise on 50m walk time ($F(3,33)=0.14$; $p=0.72$; partial eta squared=0.004).

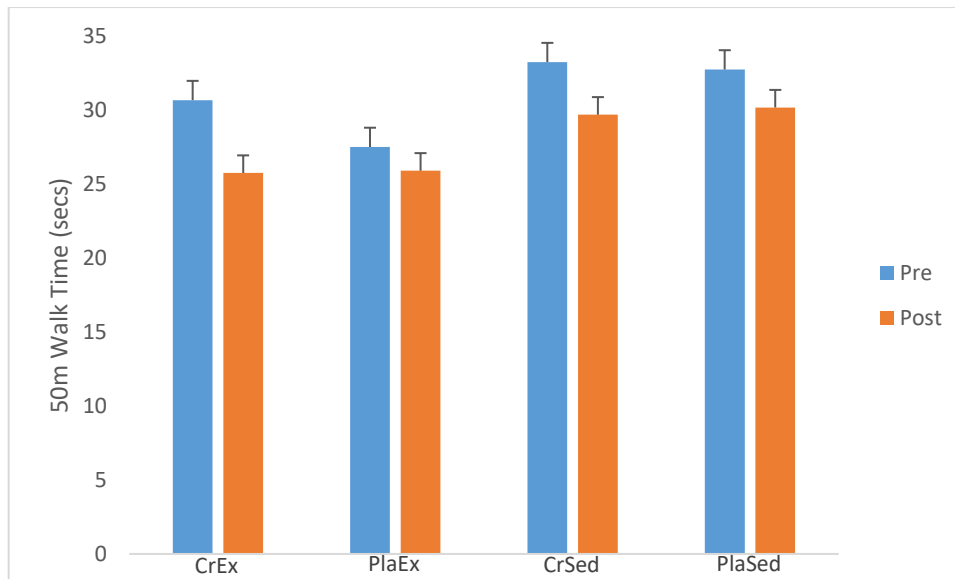


Figure 8.15: 50m walk time across groups, pre- and post-intervention. CrEx = Creatine supplement with exercise, PlaEx = Control supplement with exercise, CrSed = Creatine supplement without exercise, PlaSed = Control supplement without exercise

8.4.14 Strength and Fitness Measures

Mixed between-within ANOVA revealed that there was no statistically significant effect of supplement or of exercise on jump height, handgrip strength, flexibility, or balance (all $p > 0.05$). There was no statistically significant interaction between time and group, or between supplement and exercise, on jump height, handgrip strength, flexibility, or balance (all $p > 0.05$).

8.5 Discussion

The present study aimed to investigate the effects of a creatine supplement in combination with an exercise program on BMD and bone turnover, and functional strength, in adult women. It was hypothesised that both creatine supplementation and exercise would result in increased bone formation and decreased bone resorption, and that there would be a synergistic effect of the combination of creatine and exercise. Findings of the present study suggest that there is a trend towards increased bone formation following both creatine supplementation and exercise, although no effect on bone resorption. Results further demonstrate that creatine supplementation results in

increases in muscle strength, and that the effects of exercise on body composition measures are unaffected by creatine supplementation.

Present findings demonstrate no statistically significant effect of creatine supplementation in combination with exercise on bone formation or bone resorption. However, there was a trend towards increased bone formation following both creatine supplementation and exercise, in comparison to the control group. This increase in bone formation therefore means a change in the balance of bone turnover, in favour of formation over resorption. Creatine in combination with exercise has been shown to result in decreased bone resorption in young men and women, and in older men (Candow et al., 2008, Cornish et al., 2009). Like the present finding, this results in a change in the balance of bone turnover in favour of bone formation, although via changes in osteoclast activity rather than osteoblast activity. Up to 6 months' exercise, and exercise combined with creatine supplementation, do not result in changes in measures of bone resorption or formation in older men and women (Brose et al., 2003, Tarnopolsky, 2007). Given the differences in populations used, it is possible that populations with lower BMD, such as older women, have comparatively lower osteoblast activity, and so osteoblast activity is affected prior to osteoclast activity.

The results of the present study demonstrate that there was no significant effect of 12 weeks' creatine supplementation alone on levels of biomarkers of bone turnover, or on BMD. This is in line with previous findings that changes in biomarkers were not apparent after 3 months of supplementation. A similar trend towards increased bone formation was demonstrated after 12 months of supplementation (see Chapter 7), suggesting that the inclusion of exercise resulted in more rapid changes than supplementation alone. One years' very low dose supplementation ($1\text{g}\cdot\text{day}^{-1}$) has been shown to have no effect on bone biomarkers in osteopenic women (Lobo et al., 2015). The very low dose supplement by Lobo et al (Lobo et al., 2015), and the low dose and short duration used in the present study, could be responsible for the lack of effect; in earlier work an effect was seen after longer duration supplementation. Recommendations for future work are therefore that exercise in combination with creatine is continued for a longer duration intervention, or that a higher dose of supplementation is used during the 12 week intervention. The non-exercising population used in the present study may not have regularly undergone sufficient

physical activity to promote uptake and use of creatine; exercise increases creatine uptake into the muscle, and only into the exercised muscle (Robinson et al., 1999). In support of the present findings, creatine supplementation has been shown to have no effect on BMD in some rodent models (Alves et al., 2012, Ferreira, 2005) or in Myotonic Dystrophy type I (Tarnopolsky et al., 2004a).

In comparison to the present findings, creatine supplementation without exercise has been shown to have a beneficial effect on bone turnover in some forms of muscular dystrophy (Louis et al., 2002, Tarnopolsky et al., 2004b). Creatine supplementation has been shown to result in increased differentiation and mineralisation in osteoblast-like cells (Gerber et al., 2005), and to stimulate production of OPG, which inhibits osteoclast activity (Gerber et al., 2008). The differences in findings between the present study and Gerber et al. (Gerber et al., 2008, Gerber et al., 2005) could be explained by dose and duration; the low dose supplementation regime over a short duration may not sufficiently increase osteoblast activity and OPG production to be measurable. Longer-term supplementation has been previously shown to result in changes in bone biomarkers (see Chapter 7); short-term higher dose supplementation may also result in significant changes in bone biomarkers. Louis et al (Louis et al., 2002) and Tarnopolsky et al. (Tarnopolsky et al., 2004b) used only 3 and 4 months' supplementation (respectively), with a similar dose to the present study. This suggests that the differences in findings could be due to other factors such as diet; those who routinely consume a diet high in creatine would be less affected by supplementation (Burke et al., 2003). Analysis of the present cohort revealed no relationship between habitual creatine intake and the rate of bone turnover as calculated using NTX:ALP. However, the present cohort also demonstrated no relationship between habitual creatine intake and muscle mass, suggesting that there may not have been sufficient variation in habitual creatine intake across the cohort to stimulate physiological effects. It is also possible that other population differences such as age and gender mean that populations used in previous studies respond more quickly to creatine supplementation than the population used in the present study.

Present findings demonstrate that there was no significant effect of exercise on bone turnover; there was no significant change in the NTX:ALP ratio. There is wide variation in the literature regarding the effects of exercise on bone biomarkers. Exercise

alone has been shown not to affect bone formation in sedentary females (Hinton et al., 2012) or in active males or females (Scott et al., 2012b, Sherk et al., 2013, Whipple et al., 2004). Bone resorption has been shown to be unaffected by exercise in sedentary females (Hinton et al., 2012) or in active males or females (Bemben et al., 2015, Scott et al., 2012a), although acute responses to high intensity exercise show an increase in bone formation (Bemben et al., 2015).

Results of the present study demonstrate that 12 week's exercise does not result in changes in BMD; although a statistically significant difference was observed in BMD of the femur neck, the observed change did not exceed the LSC previously calculated (see Chapter 4), and so it could not be classed as a significant change.. Although several studies have previously reported a beneficial effect of exercise on BMD in postmenopausal women (Chow, 1987, Dalsky, 1988, Kemmler et al., 2004, Kerr et al., 2001), in premenopausal women (Dornemann, 1997, Lohman et al., 1995b, Snow-Harter et al., 1992, Vainionpaa et al., 2005), and in children and adolescents (Detter et al., 2013, Morris et al., 1997), these studies have used longer-term exercise interventions, typically a minimum of 6 months in duration. Cross-sectional studies have also demonstrated a positive effect of physical activity on BMD (Etherington et al., 1996), which suggests that long-term exercise is necessary to promote an effect. 12 weeks' exercise has been shown to have a beneficial effect on BMD of rats (Murai et al., 2015), but, due to differences in metabolism, this time frame is not necessarily comparable to a human population. 12 weeks exercise intervention appears to be too short to elicit measurable changes in BMD in this population; greater exercise intensity may result in greater changes in body composition and bone measures.

The present findings that creatine supplementation in combination with exercise does not elicit changes in BMD is supported by findings that 24 weeks' exercise training with creatine supplementation does not result in changes in BMD in postmenopausal women with osteopenia or osteoporosis (Gualano et al., 2014b) or in older men and women (Brose et al., 2003). Gualano et al (Gualano et al., 2014b) used a loading dose of $20\text{g}\cdot\text{day}^{-1}$ followed by a maintenance dose of $5\text{g}\cdot\text{day}^{-1}$, supporting the conclusion that much longer-term exercise may be necessary for inducing measurable changes in BMD. The lack of effect seen in studies of shorter duration suggests that long-term behaviour change is required to influence bone metabolism, rather than changes

occurring in the short-term and being maintained over the long-term. Creatine supplementation of $5\text{g}\cdot\text{day}^{-1}$ during 12 weeks' resistance training in elderly individuals was found not to influence BMD in comparison to placebo (Pinto, 2016). In addition, exercise intervention has been shown to be less effective at increasing BMD in populations characterised by low bone mass (Bassey et al., 1998, Nikander et al., 2010a); it is possible that creatine supplementation also has less of an effect on BMD in a population with low bone mass. Adult bone has been shown to require greater mechanical strain to elicit changes than young bone; high mechanical strains result in increased formation and decreased resorption in adult bone, whereas young bone exhibits adaptations at low mechanical strains (Razi et al., 2015). Bones of adult men exhibit a less pronounced response to a single bout of exercise than those of boys (Kish et al., 2015).

In comparison to the present findings, rodent models have demonstrated an increase in chondrocyte maturation and ossification, and increased bone mineral apposition and decreased osteoclast surface, following exercise (Thongchote et al., 2014, Troib et al., 2015); these results suggest an increase in bone formation and a decrease in bone resorption following exercise. Active males have demonstrated an increase in markers of bone formation after both acute bouts of exercise and 12 months' training (de Sousa et al., 2014, Hinton et al., 2015). Short-term (12-24 weeks) exercise training in pre- and postmenopausal women has been shown to result in increases in bone formation (Anek et al., 2011, Mohr et al., 2015, Moreira et al., 2014). A single bout of exercise has been shown to result in decreases in bone resorption in active males (Mezil et al., 2015, Whipple et al., 2004). 12 months' training in osteopenic men has been shown to result in a decrease markers of bone resorption (Hinton et al., 2015), and 24 weeks' training results in truncation of the increase in markers of bone resorption in postmenopausal women (Moreira et al., 2014).

Although Olc and ALP both measure bone formation, they show different responses over time, and to the intervention. ALP is secreted by osteoblasts during maturation of the matrix, before mineralisation of the matrix occurs. Olc is secreted by osteoblasts during mineralisation of the matrix, therefore the increase in Olc seen in the Supplement/Exercise group suggests that there is an increase in the number of mature osteoblasts, or an increase in the secretion of Olc by the mature osteoblasts. Increased

ALP in the Control/Exercise group suggests that there is an increase in the maturation of the matrix, but that this increased matrix may not undergo mineralisation.

12 months' creatine supplementation combined with exercise has been shown to result in preservation of BMD in postmenopausal women (Chilibeck et al., 2014), supporting the conclusion that a longer-term intervention is necessary to elicit measurable changes in BMD. 12 weeks' supplementation with creatine alongside exercise has been shown to result in increases in arm BMD in older men, although not in leg or whole body BMD (Chilibeck et al., 2005); given that older men typically have higher BMD than older women, this supports the supposition that creatine and exercise may be less effective at increasing or maintaining BMD in populations with low BMD; longer-term intervention may be necessary to elicit changes in these groups. The present findings of improved muscle mass and function suggest that creatine supplementation in combination with exercise may have the potential to elicit beneficial changes; given the relationship between muscle and bone through the muscle-bone unit (Schoenau, 2005), there may therefore be a resultant indirect beneficial effect on bone in adult women, a population at risk for low BMD.

Results of the present study demonstrate that exercise without supplementation results in a significant decrease in fat mass in comparison to non-exercisers, although some of the change observed in the present study may be attributable to measurement error of the DXA. Creatine in addition to exercise did not result in significant changes in body composition, suggesting that the consumption of creatine in addition to exercise may counteract some of the initial fat loss which is a result of the exercise. In support of these findings, it has been shown that creatine supplementation in combination with training does not result in additional fat-free mass gains over training alone (del Favero et al., 2012, Larson-Meyer et al., 2000). Earlier work has demonstrated that creatine supplementation alone does not result in water retention sufficient to affect the reliability of DXA measures of body composition (see Chapter 5). Findings that 12 months' creatine supplementation results in increases in muscle mass and decreases in fat mass similar to those obtained through exercise (Gualano et al., 2014b) suggests that the additional effects of exercise in combination with creatine supplementation over a short duration may result in only small additional changes in muscle and fat mass. A

longer duration or higher intensity exercise program which promotes greater changes in calorie balance may be necessary for greater changes.

In comparison to the present findings, creatine supplementation in combination with exercise has been shown to result in increases in fat-free mass and decreases in fat mass (Aguiar et al., 2013, Becque et al., 2000, Gualano et al., 2014b, Moon et al., 2013). The differences in findings could be explained by the low dose of the supplement given, and by the short duration of the exercise program. It is possible that participants undergoing the exercise intervention did not have sufficiently increased levels of physical activity due to replacing some habitual exercise with the exercise intervention. However, analysis of physical activity questionnaires demonstrated that participants' habitual physical activity levels were unchanged throughout the intervention; the exercise program served as additional physical activity, rather than replacing habitual physical activity. 12 weeks' creatine supplementation in combination with exercise in men and women aged 60-80 has been shown to result in greater increases in fat-free mass than exercise alone (Aguiar et al., 2013, Pinto et al., 2016). Differences of the present findings in comparison to Aguiar et al (Aguiar et al., 2013) and Pinto et al (Pinto, 2016) are likely to be due to differences in methodology, such as training session intensity or timing, and the population used.

The results of the present study demonstrate that there was no effect of creatine supplementation alone on measures of body composition. This is in line with previous findings that changes in body composition were not apparent after 3 months' supplementation, although an effect of creatine on fat-free mass became apparent after 12 months' supplementation (see Chapter 7). In support of these findings, creatine supplementation alone has been shown to have no effect on body mass or fat-free mass (Grindstaff et al., 1997, Larson-Meyer et al., 2000, Noonan et al., 1998). In comparison to the present findings, creatine supplementation has been shown to result in increases in body mass and fat-free mass in adult males and females (Becque et al., 2000, Green et al., 1996, Greenhaff et al., 1994, Mihic, 2000, Peeters et al., 1999, Volek, 1999, Volek and Rawson, 2004).

Earlier work found an effect after much longer duration supplementation (see Chapter 7); the present study used a low dose, short-term supplementation regime, which could explain the lack of effect in the present findings. Studies which have found an effect of

creatine supplementation on body mass have typically used a young, recreationally active population; young active participants are likely to have higher muscle mass, and so increased creatine uptake into the muscle. Longer duration supplementation has been shown to result in increased fat-free mass in postmenopausal women (Gualano et al., 2014b), suggesting the need for higher dose or longer duration supplementation to elicit a change in fat-free mass following supplementation in the present cohort.

Findings of the present study demonstrate that exercise results in a significant increase in strength in comparison to non-exercisers. Creatine supplementation in combination with exercise results in significantly greater strength gains than exercise alone. In support of the present study, findings demonstrate that creatine supplementation with exercise results in significant gains in muscle strength in young active males and females, in untrained males, and in older men and women (Becque et al., 2000, Brose et al., 2003, Cornish et al., 2009, del Favero et al., 2012, Larson-Meyer et al., 2000, Noonan et al., 1998, Peeters et al., 1999, Tarnopolsky et al., 2007). Creatine supplementation increases the body's stores of PCr, which is used in the energy production pathway in the resynthesis of ATP; higher base levels of creatine therefore allow more high-energy work to be performed, thereby increasing training adaptations through an increased training load.

In comparison to the present findings, creatine supplementation in combination with exercise has been shown to have no additional effect on muscle strength over exercise alone in recreationally active males, or in middle-aged and older men (Bemben et al., 2010, Francaux and Poortmans, 1999). The studies by Bemben et al (Bemben et al., 2010) and Francaux and Poortmans (Francaux and Poortmans, 1999) used similar creatine dosages to the present study, suggesting that the discrepancies in findings is likely to be due to gender differences between participants. The exercise protocols used may not have been of high enough intensity to elicit the uptake and utilisation of the supplemented creatine. In addition, neither Bemben et al (Bemben et al., 2010) nor Francaux and Poortmans (Francaux and Poortmans, 1999) assessed normal dietary creatine intake; given that creatine supplementation has a greater effect in individuals with a habitually low intake (Burke et al., 2003). Habitual creatine intake was calculated in the present study to assess whether habitual creatine intake changed across

the period of the intervention, but there is insufficient reference data to show whether habitual intake was high or low.

The present finding that exercise augmented by creatine supplementation resulted in increased isokinetic strength in comparison to exercise alone is comparable to findings that 12 months' supplementation resulted in similar increases in strength to exercise alone (Gualano et al., 2014b). Given the short duration of the supplementation regime, creatine supplementation over a longer time period may result in significant strength gains. Creatine supplementation has been shown to result in increases in muscle strength in trained males (Becque et al., 2000, Earnest et al., 1995), and increases in strength comparable to exercise over a 12 month period in postmenopausal women (Gualano et al., 2014b). Individuals with higher baseline muscle mass are likely to experience a greater benefit from creatine supplementation, as there is a higher uptake of creatine into the muscle, explaining the effects in young trained males. The present finding that creatine supplementation alone does not result in significant changes in fat-free mass supports this supposition that the short duration, low dose supplementation regime does not result in significant strength gains, where higher dose or longer duration supplementation may result in significant gains in both fat-free mass and muscle strength.

There is wide variation in the time at which adult women become peri- and postmenopausal. Although the duration of the present study means that it was unlikely that participants' menstrual status was altered during the study, hormonal measures such as sex hormones would have allowed menstrual status to be tracked. It is possible that some of the changes observed are due to alterations in menstrual status throughout the study. Participants who took part in the exercise sessions were progressed in the intensity of the exercise when they were able to complete each exercise with ease. However, it is possible that some participants did not progress as early as they could have done, therefore losing some effect of the exercise intensity. Although a potential limitation to the present study was adherence to the supplementation regime, all supplements were weighed and all participants had a minimum adherence of 80%. There was wide variation in blood marker measures obtained for participants in the present study, with several markers falling outside the expected range of values (Brown et al., 2009, Hannemann et al., 2013, MedScape). This could be due to participants

undergoing a change in menopausal status during the study, and so experiencing changes in hormone levels. The wide variation could suggest that some individuals had unusually high levels of certain hormones, and would therefore respond differently to the intervention. A larger sample size may have been less affected by outliers than the small sample size in the current study. Further limitations of the present study include the lack of measures of calcium excretion, and the lack of measures of intramuscular creatine. Due to the supplementation strategy used in the present study rather than a traditional “loading” and “maintenance” phase, and the rate of muscle saturation with creatine, the muscle may have taken a number of weeks to become fully saturated with creatine; there would therefore have been a shorter period of time of the exercise program during which the supplement had full effect. The supplement may therefore not have been as effective at promoting physiological benefits.

8.5.1 Conclusion

In conclusion, creatine supplementation alongside exercise has the potential to affect bone turnover in adult women. Creatine supplementation in combination with exercise results in a change in the balance of bone turnover, in favour of bone formation, demonstrating a potential for protecting against bone loss in this population. Short duration, low dose creatine supplementation in combination with exercise does not result in measurable changes in BMD or body composition, but does result in greater strength gains than exercise alone. Combined resistance and high impact exercise results in slowing of bone turnover, although not in a significant change in the balance of bone resorption to formation. Future studies should investigate the effects of a longer duration exercise program with higher dose supplement regime on bone biomarkers and BMD.

9 Synthesis of Findings

9.1 General Discussion

The introduction to this thesis discussed the increasing health concern of low bone density in an ageing population. Research into delaying and truncating the onset of disease states such as osteoporosis is therefore of increasing importance; there is increasing demand for research investigating the effects of diet and exercise on musculoskeletal health. Creatine supplementation is increasingly prevalent in sporting populations, due to its low health risks and ergogenic effects, particularly in increasing muscle mass and functional strength (Juhn and Tarnopolsky, 1998, Kreider, 2003, Kreider et al., 1998a, Poortmans and Francaux, 2000). As previously discussed, the relationship between muscle mass and BMD suggests that increased muscle mass following creatine supplementation may result in increased BMD (Schoenau, 2005). Creatine supplementation may also have a direct effect on bone metabolism, changing the ratio of bone formation to bone resorption, in favour of bone formation (Gerber et al., 2005, Gerber et al., 2008).

The thesis was designed to examine the relationship between creatine intake and musculoskeletal health, and to investigate the effects of exercise, and exercise augmented by creatine supplementation, on musculoskeletal health in adult women. To the author's knowledge, this is the first research which has investigated the effects of creatine supplementation alongside combined resistance and high impact exercise on musculoskeletal health in premenopausal women. This is a population which at high risk of developing bone-related disorders in later life, and so interventions in this population aim at preventing, rather than treating, musculoskeletal disorders.

9.1.1 Realisation of Aims

In order to achieve the aims of this thesis, a number of studies have been completed. Through completion of these investigations, the test/retest reliability of DXA and the reliability of DXA during creatine supplementation have been quantified, the relationship between creatine intake, body composition, and bone health has been assessed, and the effects of high intensity exercise, and exercise in combination with creatine supplementation, on bone health, body composition, and functional strength has been investigated. The aims of this thesis will now be briefly discussed in relation

to the results of the studies, and in relation to the limitations that were faced throughout completion of these studies. Possible implications for practice and future research will also be discussed. A schematic diagram representing the findings of the thesis is presented (9.2), which synthesises the findings of individual studies and presents the overall conclusions of the thesis.

9.1.1.1 *DXA Validity and Reliability*

The initial study conducted within this thesis allow the establishment of the reliability of the current institution's DXA scanner, and to calculate the error of the DXA measurement. Given the anecdotal evidence of creatine monohydrate supplementation causing water retention, and the reported effects of water retention on DXA reliability, study 2 then aimed to assess the effect of creatine supplementation on DXA measures of BMD and body composition. The calculation of error in study 1 allowed body composition and BMD changes in later studies to be compared to the error of the machine; body composition changes which did not exceed this error could therefore not be classed as significant.

The World Health Organisation (WHO) recommends the use of DXA to measure and to monitor changes in BMD, in order to classify individuals with low bone mineral density. Given the rapid changes which occur in BMD in ageing women, and the reported differences between different DXA machines, the change in time of BMD may be of more clinical significance than one-off measures of BMD. It is therefore important that the test/retest validity of DXA measures be sufficient that the change in BMD over time can be accurately tracked. DXA is sufficiently reliable that changes in BMD over time can be monitored on a single machine.

Dual Energy X-ray Absorptiometry is often used as a method for measuring and monitoring changes in body composition, as it presents a quick and easy method of accurately calculating fat-free mass and fat mass. DXA is frequently used in sporting populations, to establish changes in fat-free mass and in fat mass throughout training and sporting seasons. The test/retest reliability of DXA must therefore be established in order to accurately track changes in body composition over time. The ISCD gives guidelines regarding conducting precision studies, and the levels of acceptable error for DXA scanners and operators. The precision of the current DXA was sufficient to

accurately track changes over time, and the error of the machine was calculated; changes in body composition and BMD need to exceed the calculated error of the DXA in order to be classed as significant changes.

High dose creatine supplementation did not result in significant changes in body composition measures. Creatine supplementation at $20\text{g}\cdot\text{day}^{-1}$ for 7 days does not cause sufficient water retention to affect measures of fat-free mass or of fat mass by DXA. DXA can therefore be used as a method for monitoring changes in body composition during a period of creatine supplementation. Given the increasing prevalence of creatine supplementation in sporting populations, this finding allows the monitoring of changes in fat-free mass and fat mass throughout training and sporting seasons.

9.1.1.2 *Creatine Effects on Bone Health*

Creatine supplementation has been associated with increased muscle mass and performance in a variety of populations. Increased muscle mass and increased incidence of muscle contraction results in increased pull of muscle on the bone at the sites of attachment, and therefore increased stimulation for bone growth at these sites. Creatine may also have the potential to stimulate bone growth through a direct effect on osteoblast and osteoclast function. The effects of creatine supplementation on bone health in premenopausal women, a population at high risk of developing osteoporosis, has not been conclusively determined. The present thesis investigated the effect of creatine supplementation on BMD and blood markers of bone resorption and formation.

Creatine supplementation in young boys with muscular dystrophy, and in some experimental models of osteoporosis, results in increased markers of bone formation and decreased markers of bone resorption (Antolic et al., 2007, Louis et al., 2002, Tarnopolsky et al., 2004b). The present thesis demonstrated no significant relationship between habitual creatine intake and BMD (Study 3: The Relationships Between Habitual Creatine Intake, Physical Activity, BMD, and Muscular Strength), and there appears to be no significant effect of creatine supplementation on BMD, or on markers of bone health (Study 5: The Effects of Creatine Monohydrate Supplementation and Exercise on Musculoskeletal Health in Women). Nor did findings demonstrate a significant effect of low-dose, long-term creatine supplementation on BMD or on blood markers of bone formation or resorption (Study 4: The Effects of Creatine

Monohydrate Supplementation on Musculoskeletal Health in Women). A low-dose, 12-week creatine supplementation did not affect BMD or on blood markers of bone formation or resorption (Study 5: The Effects of Creatine Monohydrate Supplementation and Exercise on Musculoskeletal Health in Women). There was no significant effect of long-term creatine supplementation, or exercise augmented with creatine supplementation, on the rate of bone turnover.

Previous findings have demonstrated the different effects of exercise on individuals with high or low bone density, suggesting that the findings of the present thesis could be a result of individuals in the experimental studies having a high initial bone density. Given that individuals with low bone density are more responsive to exercise programs designed to improve bone density, individuals with high bone density may be less responsive to creatine supplementation. Despite previous findings that low-dose creatine supplementation may influence bone and muscle metabolism (Candow, 2008), it is also possible that the dosage used in the present studies were too low to stimulate a physiological response in this population; higher dose supplementation would result in a greater increase in muscular PCr levels and in subsequent associated changes, as well as in greater stimulation of osteoblast and inhibition of osteoclast function; although findings have shown that short-term creatine loading results in similar increases in creatine stores to long-term, low-dose supplementation (Hultman, 1996), a higher dose may stimulate a greater physiological response due to a greater increase in muscle PCr. However, a higher dose regime may prove to be less palatable and so less sustainable in this population. Muscle also has a finite capacity for PCr storage, suggesting the need for future studies to include measures of intramuscular PCr, to calculate PCr saturation and maximal beneficial supplementation protocols.

9.1.1.3 *Creatine Effects on Muscle Mass and Strength*

Creatine supplementation has been associated with increased muscle mass and strength, and decreased fat mass, when accompanying an exercise training regime (Camic et al., 2014, Kreider, 2003, van Loon et al., 2003). Creatine supplementation increases the body's stores of PCr, which is used in the production of energy for the working muscle. The effects of creatine supplementation on body composition without accompanying exercise has not been conclusively determined, although habitual physical activity during daily activities could cause creatine uptake by the working muscle and therefore

result in similar changes. The present thesis investigated the effects of creatine supplementation on body composition, specifically on fat-free mass as measured by DXA, and on functional muscle strength.

The present thesis did not report a relationship between habitual creatine intake and muscle mass and strength, but an effect of creatine supplementation on muscle. There was no significant effect of creatine supplementation on muscle mass and strength, although there was a significant increase in isokinetic leg strength following short-term creatine supplementation. It is possible that participants in the present studies did not undergo sufficient physical activity during daily life to stimulate the uptake of creatine into the muscle. However, when physical activity was used as a controlling factor, there was still no effect of creatine supplementation on body composition measures.

Given the paucity of studies which have investigated the effects of creatine supplementation in this population without accompanying exercise, the present findings fill a gap in the literature. Present findings imply that creatine supplementation without exercise in this population is insufficient to stimulate changes in body composition, although there were significant gains in isokinetic leg strength. These gains in leg strength following supplementation show that there is the potential for creatine supplementation to have ergogenic effects without accompanying exercise, although suggest that the dose used in the present study may be too low to result in changes in fat-free mass, and in overall functional strength. The sample size used in investigation of long-term creatine supplementation was too low to give sufficient statistical power to the statistical tests performed. Larger sample sizes would be necessary to obtain results with a greater statistical power; the present results therefore give pilot data to show the need for a larger study to investigate the effects of creatine supplementation on muscle wastage in an older population. Present findings are insufficient to result in recommendations of creatine supplementation alone for increasing muscle mass and strength, although findings do show the potential of creatine supplementation for improving muscle strength without associated exercise.

9.1.1.4 *Exercise and Creatine Effects on Bone*

Exercise is associated with increased bone density in younger as well as in older individuals (Warden and Mantila Roosa, 2014, Warden et al., 2014); exercise promotes

bone formation through stimulating osteoblast function, and inhibiting osteoclast function. Bone adaptation is also increased through exercise, via increased stresses on the bone through impact, as well as through increased pull of muscle on bone. Creatine supplementation in combination with exercise has been associated with greater changes in body composition than exercise alone (Kreider, 2003, Rawson and Volek, 2003). Physical activity promotes creatine uptake into the working muscle; in turn, increased PCr stores allow more high intensity work to be performed, therefore promoting the physiological adaptation to exercise. The present thesis investigated the effects of exercise, and exercise combined with creatine supplementation, on BMD and on blood markers of bone resorption and formation.

Although findings demonstrated no significant effect of exercise on BMD, there was a significant decrease in bone resorption following exercise without creatine supplementation, suggesting a protective effect against bone loss following exercise. Bone resorption markers respond more quickly to changes in bone turnover than bone formation markers; it is therefore possible that a longer duration intervention may also result in changes in markers of bone formation. Significant, measurable changes in BMD take a minimum of 6-9 months to occur due to bone turnover being a slow process, and therefore the changes in bone resorption could lead to changes in BMD over time. There was no augmentative effect of creatine on bone turnover beyond exercise alone, suggesting that creatine supplementation does not contribute to bone health in this population. It is possible that the frequency and intensity of the exercise intervention used in the present thesis was insufficient to challenge or deplete the PCr system, therefore supplementation with creatine would not result in increased adaptations to exercise. Previous literature has reported that long-term, low-dose creatine supplementation does not result in changes in BMD in postmenopausal women (Gualano et al., 2014b), lending support to the present findings that creatine supplementation does not influence bone health in the present population.

Present findings are explained by Wolff's Law and the Mechanostat theory; bones are able to adapt stresses placed upon them. High impact exercise puts stress on the bones, resulting in increased bone formation and decreased bone resorption through inhibition of osteoclast function; over time, this increased bone formation could result in significantly increased BMD. Given that present findings show increased bone

formation in adult women, there could then be an effect of increased peak bone density over a longer duration; this increased peak bone density would mean that individuals were at less risk for developing bone degenerative disorders such as osteoporosis.

9.1.1.5 *Exercise and Creatine Effects on Muscle*

Creatine supplementation and exercise have been demonstrated to result in increased muscle mass and strength gains beyond exercise alone. Creatine supplementation increases the body's stores of PCr, the energy source for the working muscle. This increased PCr allows a greater amount of high intensity work to be performed before fatigue sets in, therefore allowing greater training adaptations. Creatine supplementation has been shown to result in increased muscle mass and strength above exercise alone in a variety of populations. The present thesis investigated the effects of exercise, and exercise combined with creatine supplementation, on fat-free mass as measured by DXA, and functional muscle strength.

Findings of the present thesis show that there is increased fat-free mass, and decreased fat mass, following a short-term exercise intervention. Increased functional strength is observed following a short-term exercise intervention. Muscle mass and strength are correlated with BMD, suggesting that a long-term exercise intervention may result in improved bone health in accordance with Wolff's Law. Findings of the present study with regards exercise and body composition show that individuals with low levels of physical activity can have significant alterations in fat-free mass, in fat mass, and in muscle strength, following short-term exercise programs.

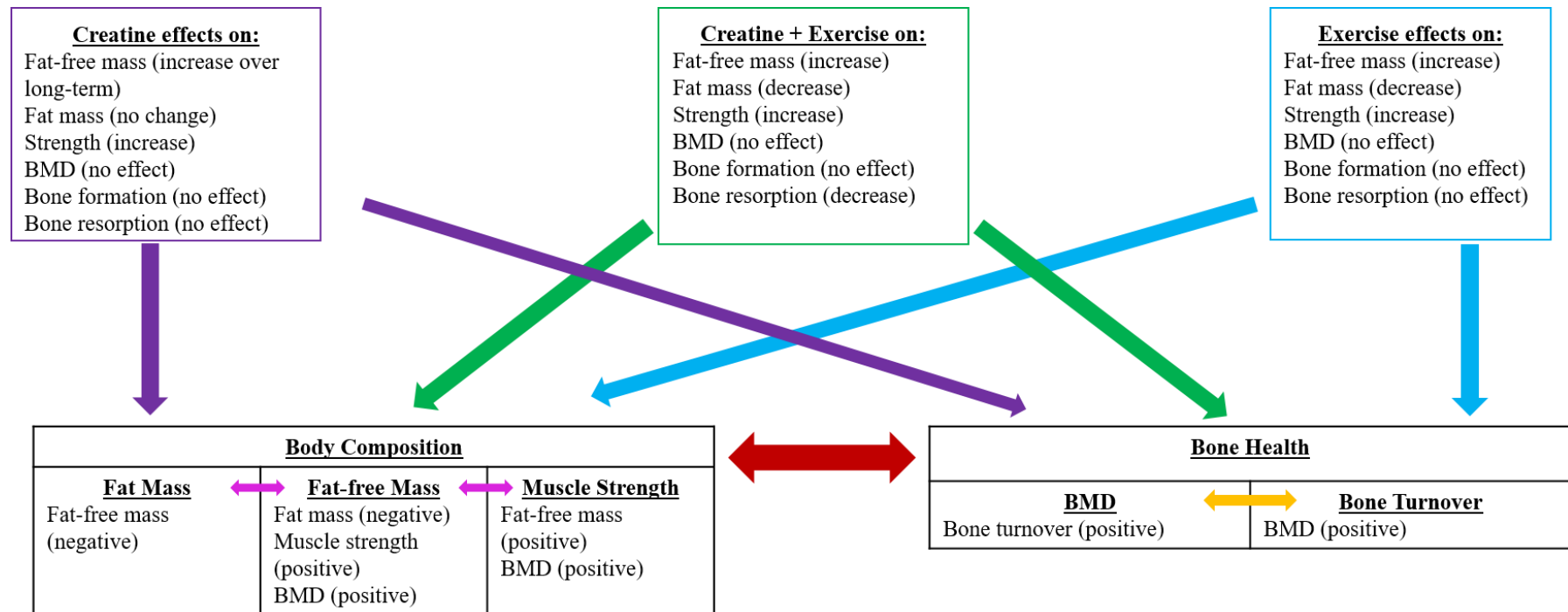
Creatine supplementation is widely used in athletic populations as an ergogenic aid, to complement training regimes. Present results do not show an augmentative effect of creatine supplementation on fat-free mass or muscle strength beyond exercise alone, suggesting that low-dose creatine supplementation is insufficient to stimulate additional changes above those gained through exercise. The training program and supplementation protocol used may also have been of too short duration for significant gains. These findings do not support the use of low-dose creatine alongside a short duration training program, but suggest that the strength gains from the short duration training program are not improved through the addition of low-dose creatine

supplementation; the muscle may not be sufficiently saturated with creatine to promote physiological effects.

Previous findings have demonstrated an augmentative effect of creatine supplementation alongside exercise on muscle mass and strength. The present findings therefore suggest that the low-dose supplement used in the present thesis was insufficient to stimulate changes in body composition, supporting the supposition that the tissues may have been insufficiently saturated with creatine. This could also explain why there were no observed effects of creatine supplementation on bone health; higher doses may be necessary to stimulate physiological changes in the present population.

A limitation of the studies performed in the thesis is that there was no measure of creatine uptake, or the response of PCr levels to the creatine supplementation. There was also no measure of muscular creatine levels, to assess the amount of creatine taken up into different working tissues. Measurements of urinary creatinine, or use of creatine tracers to measure intramuscular creatine levels, would allow assessment of the amount of creatine taken up by the body's tissues.

9.2 Schematic representing the overall findings of the thesis; the relationships between creatine, exercise, body composition, and bone health



9.3 Implications and Conclusions

There is increasing interest in improving musculoskeletal health in ageing individuals. The majority of interventions aim at treatment of disease states, rather than cure. Premenopausal women are a population particularly at risk for developing osteoporosis and related disorders, and so the present thesis aimed to investigate methods of improving bone health in this population. To the author's knowledge, this is the first research which has investigated creatine and exercise in this population.

In practice, findings of the present thesis do not result in recommendations for low-dose creatine supplementation to protect bone density, or to improve bone health in premenopausal women. High impact exercise may have the potential to positively influence bone health by decreasing bone resorption, although no significant change in the rate of bone turnover was observed. These results therefore do not allow recommendations for the use of short-term, high-intensity mixed aerobic and resistance exercise to improve bone density, however it may have the potential to improve bone health over time. Longer-term exercise programs, or programs using different exercise modes such as resistance exercise alone, may have the potential to improve bone turnover. The present findings that impact exercise results in decreased bone resorption demonstrate that there is a potential for long-term exercise programs to improve bone health in adult women, although there was no effect on the rate of bone turnover. This conclusion is supported by previous work demonstrating the positive influence of exercise on bone health in a variety of populations.

Exercise results in changes in body composition, both increases in fat-free mass and decreases in fat mass, and accompanying increases in muscular strength in adult women. Muscle mass and strength are both predictors of bone health, yet it is unclear whether muscle influences bone growth, whether bone influences muscle growth, or whether both bone and muscle are influenced by a third factor. Despite this uncertainty, a relationship between muscle and bone is currently demonstrated, with decreases in the mass and quality of both in ageing individuals. Increased muscle mass and strength may therefore result in increased bone formation and increased bone density over a longer duration exercise program. Muscle mass and strength are also correlated with independence, mobility, and quality of life in older individuals, as well as decreasing risk factors for a number of disease states. Regular exercise throughout life is therefore

important for maintaining a healthy lifestyle and decreasing the risk of developing a variety of disease states such as diabetes, coronary heart disease, and cardiovascular disease, as well as maintaining quality of life.

Findings of the experimental studies conducted in this thesis demonstrate no significant beneficial effect of creatine supplementation on bone health in premenopausal women. However, there was also no demonstrated negative effect of creatine supplementation on bone health, in contrast to findings that protein consumption may have a degradative effect on bone through an effect on calcium balance (Bushinsky et al., 2001, Ginty, 2003, Kraut et al., 1986, Sutton et al., 1979). It is possible that the lack of positive findings with regards to creatine supplementation could be due to the dosage used in the present studies; low-dose supplementation may be insufficient to significantly influence bone turnover, or a longer duration supplementation protocol may be necessary to elicit changes from a low-dose supplement regime. Previous research has found a positive effect of creatine supplementation on bone health in individuals suffering from degenerative disorders (Louis, 2002; Tarnopolsky, 2004). It is therefore possible that the lack of effect seen in the present thesis is due to the population used; the mechanism of bone loss during ageing may differ from that seen in degenerative disorders. Given the population of adult women used in the present thesis, there may have been changes in menstrual status or function which would have affected bone turnover; menstrual status was assessed at the start of each study, but not at the end, meaning that changes in menstrual status may have occurred. In addition, although no participants gave reports of menstrual dysfunction, dysfunction may have occurred throughout the completion of studies.

Creatine supplementation in combination with exercise does not result in significant additional changes in body composition in premenopausal women. There were no additional positive gains on fat-free mass or muscle mass, although there is a positive effect on isokinetic and functional muscle strength. The lack of positive findings with regards creatine supplementation and muscle mass and strength in the current thesis could be due to the low-dose supplement and the duration used. This lends support to the supposition that the lack of effect of creatine on bone could be due to the low dose supplement used.

Creatine supplementation is increasingly used in athletic populations due to its reported ergogenic effects. These effects were not apparent in the present thesis, apart from in increasing isokinetic leg strength. The potential of creatine supplementation for influencing muscle strength is therefore demonstrated, although higher doses may be necessary to increase functional strength measures and body composition. Present findings suggest that use of low doses of creatine supplementation are insufficient for improving body composition and functional strength in exercising populations; higher doses may be necessary to elicit changes in adult women. Throughout the present thesis, no participants reported adverse effects following creatine supplementation. This supports previous findings that creatine is a safe supplement for human consumption, and expands the previous findings that creatine supplementation is a safe supplement in a variety of populations.

Despite previous research which has demonstrated a direct effect of creatine supplementation on bone formation *in vitro* (Gerber, 2005; Gerber, 2008), the present findings that creatine supplementation has the potential to influence the adaptations to exercise of muscle, but not bone, are supported by previous findings that creatine supplementation results in changes in fat mass and fat-free mass but not bone mass *in vivo* (Gualano, 2014). These findings suggest that creatine may be taken up by the body into muscle in preference to bone; changes in bone could therefore occur following a longer supplement protocol, through indirect effects on bone mediated by the muscle. Higher doses of supplementation may be necessary to stimulate uptake into the bone, or there may be other physiological mechanisms which prevent uptake.

9.3.1 Recommendations for further research

There is a potential for future research in this area. Future studies could investigate the effects low-dose, long-term supplementation in a larger cohort, and the effects of higher-dose, long-term supplementation with creatine monohydrate. However, given the large drop-out rate encountered in the present research during longitudinal studies, these studies may not be feasible, especially if research hopes to result in clinical implications. Recommendations in a general, non-monitored population would be vulnerable to lower adherence, and therefore studies investigating long-term supplementation may not be applicable to a general population.

Although there is a potential for creatine to influence bone mechanisms at a molecular level, and in some individuals with clinical bone disorders, there appears to be no effect at *in vivo* testing in healthy adults; the population used in the present thesis did not present disordered bone states. There may therefore be other mechanisms to take into account which reduce its efficacy during exercise in adult humans. Research into higher creatine dosages would need to consider practicalities and economic cost; the large drop-out rate seen during 12 months' supplementation means that a very large cohort would be needed to sustain a high dose supplementation regime. Future studies investigating creatine supplementation should include measures of urinary creatinine and of intramuscular creatine, in order to assess the uptake of creatine into the system. Future studies should also investigate methods of assessing the amount of creatine taken up into the muscle, in order to calculate the creatine uptake into different working tissues.

Postmenopausal women exhibit lower bone density than premenopausal women; future studies should therefore investigate the effects of creatine supplementation and exercise programs in this population, as the lower initial bone density may be more responsive to a low supplement dose. Future studies should also include measures of hormones such as oestrogen, and assessment of changing menstrual function or dysfunction, to assess the changing bone turnover process. Measurement of baseline creatine levels may also allow responders and non-responders to be identified. Different exercise modes and a longer exercise program should also be investigated, as longer duration exercise or different exercise modes may promote greater physiological adaptations. It is possible that during the exercise program, the increased muscle use allowed the creatine supplement to have a greater uptake; increasing the creatine dose throughout the exercise program may therefore result in greater uptake of the creatine into the working tissues, and so stimulate a greater physiological response, allowing investigation of the changes in resorption rate.

Given the demonstrated link between muscle and bone, future research should also focus on improving bone through improved muscle mass and quality. The possible preferential uptake of creatine into muscle should be investigated using creatine trackers to assess the amount of creatine taken up into the muscle. This would allow further investigation into maximal doses taken up into the working muscle, and

alongside clearance measures would allow calculation of the amount of creatine taken into the muscle and into other biological tissues.

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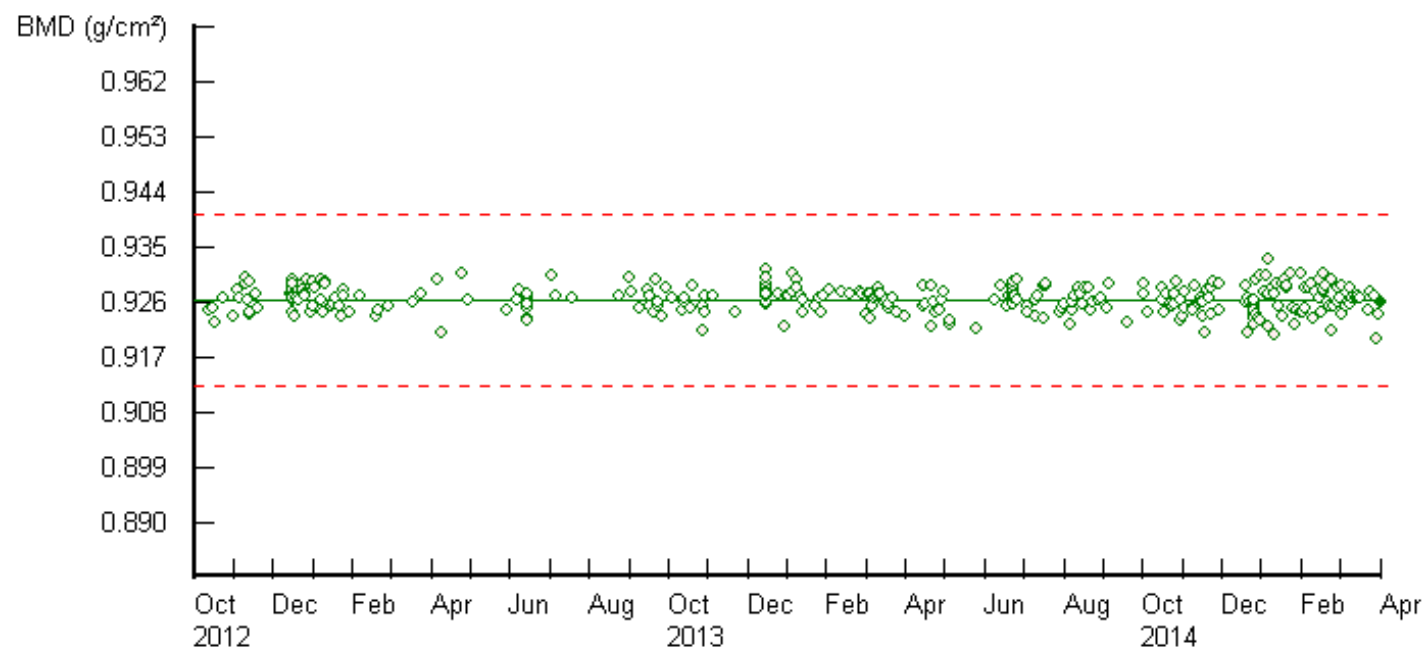
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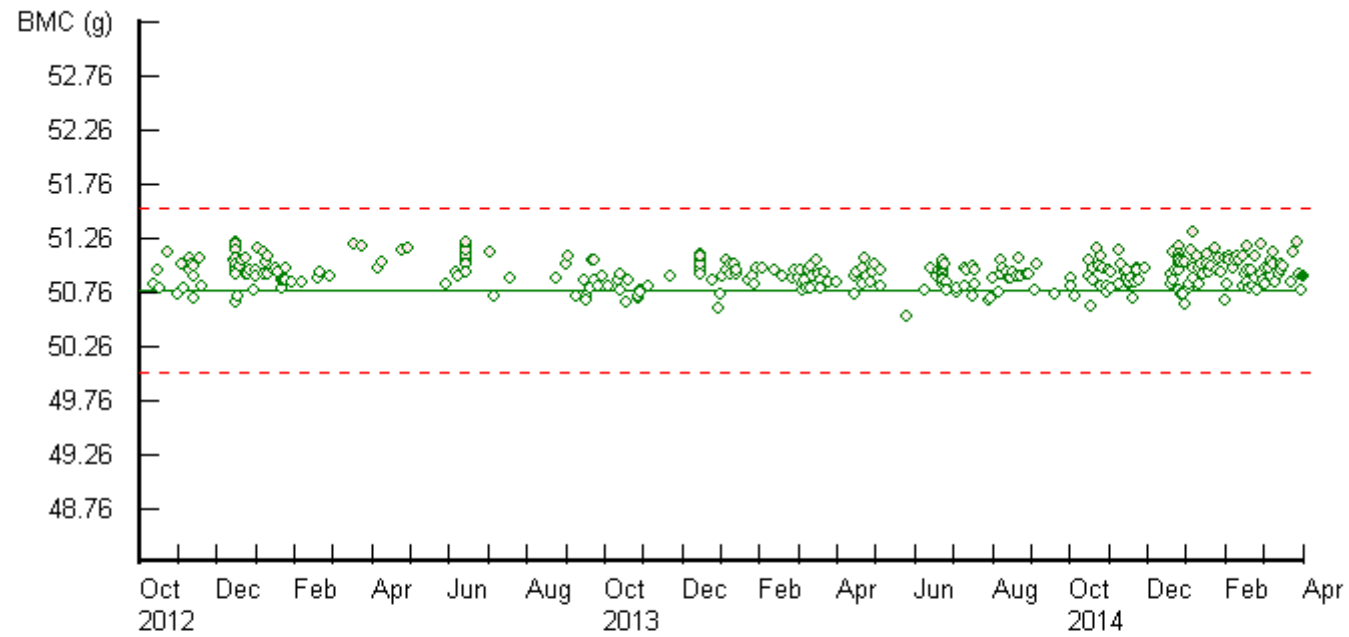
10 Appendix A.

1. QA of Bone Mineral Density (BMD) Measurement.



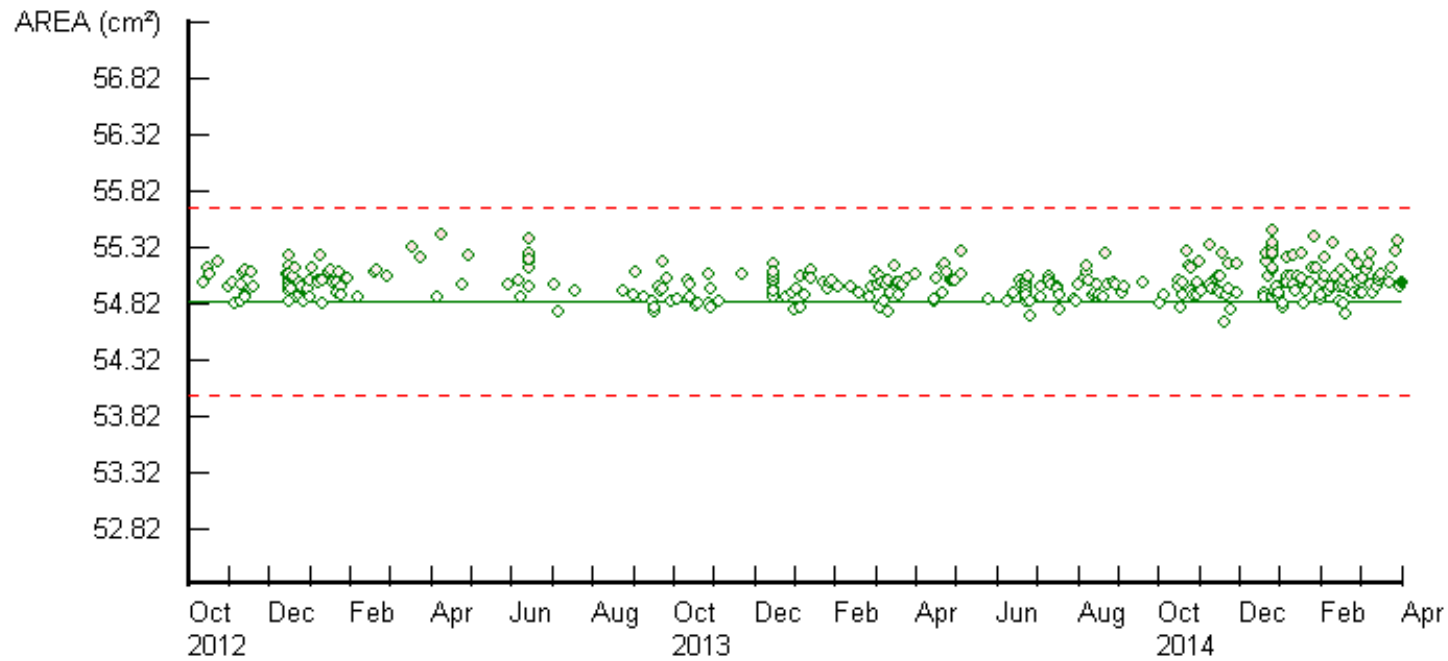
Setup	Reference Values	Plot Statistics
a Lumbar Spine phantom #21532 System S/N: 84117	Limits: $\pm 1.5\%$ of mean Mean: 0.926 (g/cm ²) SD: 0.001 (g/cm ²)	Number of Points: 334 Mean: 0.926 (g/cm ²) SD: 0.002 (g/cm ²) CV: 0.231 %

2. QA of Bone Mineral Content (BMC) Measurement



Setup	Reference Values	Plot Statistics
a Lumbar Spine phantom #21532 System S/N: 84117	Limits: $\pm 1.5\%$ of mean Mean: 50.764 (g) SD: 0.056 (g)	Number of Points: 334 Mean: 50.927 (g) SD: 0.128 (g) CV: 0.252 %

3. QA of Bone Area Measurement.



Setup	Reference Values	Plot Statistics
a Lumbar Spine phantom #21532 System S/N: 84117	Limits: $\pm 1.5\%$ of mean Mean: 54.820 (cm ²) SD: 0.069 (cm ²)	Number of Points: 334 Mean: 54.993 (cm ²) SD: 0.143 (cm ²) CV: 0.261 %

11 Appendix B Participant information sheet 1



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622619)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, 01970 628681)

Dr. Rhys Thatcher (ryt@aber.ac.uk, 01970 628630)

Fergus Guppy (feg10@aber.ac.uk, 01970 622282)

Test-Retest Validity of Dual X-Ray Absorptiometry (DXA) Combined Whole-body, Hip and Spine scans

Participant Information Sheet

As well as giving useful information on your body composition (body fat mass, muscle mass, and percent body fat) the DXA scan provides feedback on the density of your bones, giving an indication of fracture and osteoporosis risk.

Only one visit will be required to the Sport and Exercise Science department. The procedure will involve lying still on a slow moving bed, while minute quantities of x-rays (equivalent to 10 days radiation exposure from natural background sources or 1 Chest x-ray) are passed through the body. We will perform a whole body scan, a proximal femur (hip) scan and a lumbar (lower spine) scan. There will then be a 5 minute break before the process is repeated. The whole visit including briefing, analysis and a debrief will take no more than 60 minutes, with the whole body scan lasting less than 2 ½ minutes and the proximal femur and lumbar spine scans lasting approximately 10 seconds each.

You will be asked to remove any jewellery and metal (e.g. belts, zips and under wired bras) during the test, as metal will affect the measurement; if these are not removable it may still be possible to participate, but please inform the investigator. You are also asked to inform the tester of any metal implants that you have in your body (i.e. pins in bones). You should also inform the investigator if you are pregnant or if there is a chance you may be pregnant before you undergo the tests. In addition to the scans you will be asked to complete a quick medical criteria questionnaire regarding your capacity to undertake a DXA scan and to ensure that you are well within the legal recommendations for annual radiation doses. Height and weight will also be measured.

All information provided or obtained will be treated with utmost confidentiality, and no information that could lead to your identity will be disclosed in any reports on the project, or to any other party. If any results are found which are outside normal values, you will be referred to the relevant medical practitioner. All data collected will be stored securely for an

indefinite period of time with a minimum of 6 years for use in research publications; however any data published will be kept anonymous. The data collected may also be used in further research projects and/or by other researcher which have ethical approval.

All participants have the right to withdraw from the study at any time without prejudice to access of services that are already being provided or may subsequently be provided to the participant. If you are unhappy with any part of your participation you can complain about the study. To complain about the study, you need to write to: The secretary of the Aberystwyth University Ethics Committee for Research Procedures, Deans' Office, Cledwyn Building, Aberystwyth University.

Please Note:

You should inform the investigator if you are pregnant or if there is a possibility that you may be pregnant before you undergo the tests.

12 Appendix C. Informed consent form 1



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622619)

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Dr. Rhys Thatcher (ryt@aber.ac.uk, 01970 628630)

Fergus Guppy (feg10@aber.ac.uk, 01970 622282)

Test-Retest Validity of Dual X-Ray Absorptiometry (DXA) Combined Whole-body, Hip and Spine scans

Informed Consent Form

I have read and understood the information sheet and have completed the medical criteria questionnaire. The information provided on the questionnaire is correct to the best of my knowledge and I understand it will be treated in the strictest confidence. The experimenter has fully explained the purpose of the experiment and the possible risks involved. I understand that if there are any results found outside the normal range of values I will be referred to the relevant medical practitioner. I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, and that I can withdraw at any stage of the project without being penalised or disadvantaged in any way. I will adhere to the instructions of the experimenter regarding safety before, during and after experimentation.

I,.....

(Participant's full name)*

hereby volunteer to participate in experimental work as a participant.

Signed Date

(Participant)

I,

(Investigator's full name)*

certify that the details of this procedure have been fully explained and described in writing to the subject named above.

Signed Date

(Investigator)

13 Appendix D. DXA radiation exposure questionnaire

Criteria for Body Composition and Bone Health Assessment using Dual-energy X-ray Absorptiometry

All information provided on this form will be kept confidential

Name of GP _____

Name of GP surgery _____

All participants must be excluded if the answer is yes to any of the following:

Please Circle the answer that applies to you.

Are you younger than 18 years of age? **YES** **NO**

Are you / or do you think you could you be pregnant? **YES** **NO**

Date of **start** of last period (if applicable): _____

Have you had or are you currently undergoing radiation therapy? **YES** **NO**

Have you had a barium meal in the last 48 hours? **YES** **NO**

To monitor your exposure to x-ray radiation, please answer the following:

Have you had any x-rays for medical purposes in the last 12 months? **YES** **NO**

If you answered yes to the previous question, please provide detail of the number and type of scans you had conducted.

Have you had any DXA scans (not for medical reasons) from any institution other than at Aberystwyth University, Sport and Exercise department in the last 12 months?

YES **NO**

If you answered yes to the previous question, please provide detail of the number and type of scans you had conducted.

The following will not affect your ability to undertake a scan however, they may affect the interpretation of your results.

Please Circle the answer that applies to you.

Do you have a pacemaker	YES	NO
Do you have any surgical pins or plates	YES	NO
Do you have any synthetic joints e.g. hip replacement	YES	NO

For use by the operational user:

How many whole body scans have been conducted in the last 12 month period?

How many proximal femur scans have been conducted in the last 12 month period?

How many AP spine scans have been conducted in the last 12 month period?

How many other scans have been conducted in the last 12 month period?

Please provide what type of scan was conducted:

What is the total effective dose to whole body received during the last 12 months?

Number of whole body scans _____ x 0.008 mSv = _____

Number of proximal femur scans _____ x 0.040 mSv = _____

Number of AP spine scans _____ x 0.040 mSv = _____

Other _____ x _____ mSv = _____

Total effective dose for past 12 months = _____

Dose the total effective dose for the past 12 months exceed 1mSV?

YES

NO

If yes was ticked for the last question, do not conduct any further scans on the participant.

14 Appendix E. Participant information sheet 2



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622078)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, **01970 628681**)

Dr. Alex Gonzalez de Agüero (alg28@aber.ac.uk, **01970 628560**)

The Effects of Creatine Monohydrate Supplementation on DXA Scans of Bone Mineral Density

Participant Information Sheet

The current study is a 2-week study, involving consumption of 20g of a nutritional supplement daily for the course of the study. The supplement will be either a creatine monohydrate supplement or a micro-crystalline cellulose placebo. Neither creatine monohydrate nor micro-crystalline cellulose has been found to have any adverse side-effects.

The initial visit to the Department will involve anthropometric measures (height and body mass) being taken, followed by DXA scan and a bioelectrical impedance analysis (BIA) test. Participants will then be assigned to either the creatine group or to the placebo group, although they will not know which group they are in.

All participants will be asked to visit the Department three times, each seven days apart. At each visit, follow-up measures will be taken; this involves repeats of the height and weight measures, DXA scans, and BIA test. At the final visit study participants will be given their results from throughout the study and will be told which supplement they have been taking.

The DXA procedure will involve lying still on a slow moving bed, while minute quantities of x-rays (equivalent to 10 days radiation exposure from natural background sources or 1 Chest x-ray) are passed through the body. We will perform a whole body scan, a proximal femur (hip) scan and a lumbar (lower spine) scan. The whole body scan lasts less than 2 ½ minutes and the proximal femur and lumbar spine scans last approximately 10 seconds each. You will be asked to remove any jewellery and metal (e.g. belts, zips and under wired bras) during the test, as metal will affect the measurement; if these are not removable it may still be possible to participate, but please inform the investigator. You are also asked to inform the tester of any metal implants that you have in your body (i.e. pins in bones). You should also inform the investigator if you are pregnant or if there is a chance you may be pregnant before you undergo the tests. In addition to the scans you will be asked to complete a quick medical criteria questionnaire regarding your capacity to undertake a DXA scan and to ensure that you are well within the legal recommendations for annual radiation doses. Height and weight will also be measured.

The BIA test involves lying still, while self-adhesive electrodes are placed on the right hand and right foot. A small electrical current will pass between the electrodes; this current is usually undetectable.

Benefits

As well as giving useful information on your body composition (body fat mass, muscle mass, and percent body fat) the Dual Energy X-Ray Absorptiometry (DXA) scan used provides feedback on the density of your bones, giving an indication of fracture and osteoporosis risk.

Risks

There are risks associated with radiation and x-rays. However, these risks are minimal due to use of very low-dose scans; the scans used in the current study result in an amount of radiation exposure equivalent to less than 1 chest x-ray. Your radiation exposure will be monitored to make sure that you are within a safe range.

Confidentiality

All information provided or gathered will be treated with complete confidentiality, and no information that could lead to your identity will be revealed in any reports on the project, or to any other party. If any results are found which are outside normal values, you will be referred to a specialist at Bronglais Hospital. All data collected will be stored securely for an indefinite period of time with a minimum of 6 years for use in research publications; however any data published will be kept anonymous. The data collected may also be used in further research projects and/or by other researchers which have ethical approval.

Complaints and Withdrawal

You have the right to withdraw from the study at any time until publication of the results. You also have the right to withdraw at any time during testing. You have the right to withdraw without giving reason, without prejudice to access of services that are already being provided or may subsequently be provided to you. If you would like to withdraw at any time simply inform Jo Worthington (contact details above). If you are unhappy with any part of your participation you can complain about the study.

15 Appendix F. Informed consent form 2



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622078)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, **01970 628681**)

Dr. Alex Gonzalez de Agüero (alg28@aber.ac.uk, **01970 628560**)

The Effects of Creatine Monohydrate Supplementation on DXA Scans of Bone Mineral Density

Informed Consent Form

I have read and understood the information sheet and have completed the medical criteria questionnaire. The experimenter has fully explained the purpose of the experiment and the possible risks involved. I understand that if there are any results found outside the normal range of values I will be referred to the relevant medical practitioner. I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, I can choose to stop at any time, and that I can withdraw at any stage of the project until publication of the results without being penalised or disadvantaged in any way. I will adhere to the instructions of the experimenter regarding safety before, during and after experimentation.

	Initial Showing Consent
International Physical Activity Questionnaire	
3 Day Food Diary	
DXA Scans	
Contact Dr. Jones	
BIA	
Supplementation 10g, 2x day	

I,.....

(Participant's full name)*

hereby volunteer to participate in experimental work as a participant.

Signed

Date

(Participant)

I,

(Investigator's full name)*

certify that the details of this procedure have been fully explained and described in writing to the subject named above.

Signed

Date

(Investigator)

16 Appendix G. iPAQ (short)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRES

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED

FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken in 12 countries (14 sites) across 6 continents during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages. IPAQ is suitable for use in regional, national and international monitoring and surveillance systems and for use in research projects and public health program planning and evaluation. International collaboration on IPAQ is on-going and an international prevalence study is under development.

Using IPAQ

Worldwide use of the IPAQ instruments for monitoring and research purposes is encouraged.

It is strongly recommended, to ensure data quality and comparability and to facilitate the development of an international database on health-related physical activity, that

1. no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments,
2. if additional questions on physical activity are needed they should follow the IPAQ items,
3. translations are undertaken using the prescribed back translation methods (see website)
4. new translated versions of IPAQ be made available to others via the web site to avoid duplication of effort and different versions in the same language,
5. a copy of IPAQ data from representative samples at national, state or regional level be provided to the IPAQ data storage center for future collaborative use (with permission) by those who contribute.

More Information

Two scientific publications presenting the methods and the pooled results from the IPAQ reliability and validity study are due out in 2002.

More detailed information on the IPAQ process, the research methods used in the development of the IPAQ instruments, the use of IPAQ, the published papers and abstracts and the on-going international collaboration is available on the IPAQ web-site. www.ipaq.ki.se

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

*NOTE: EXAMPLES OF ACTIVITIES MAY BE REPLACED BY CULTURALLY RELEVANT EXAMPLES WITH THE SAME METS VALUES (SEE AINSWORTH *ET AL.*, 2000).*

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. This is part of a large study being conducted in many countries around the world. Your answers will help us to understand how active we are compared with people in other countries.

The questions are about the time you spent being physically active in the last 7 days. They include questions about activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Your answers are important.

Please answer each question even if you do not consider yourself to be an active person.

THANK YOU FOR PARTICIPATING.

In answering the following questions,

1. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.
2. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

1a. During the last 7 days, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling,?

Think about *only* those physical activities that you did for at least 10 minutes at a time.

_____ days per week ☐

or

- none

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities?

_____ hours _____ minutes

2a. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week ☐

or

- none

2b. How much time in total did you usually spend on one of those days doing

moderate physical activities?

_____ hours _____ minutes

3a. During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____ days per week ☐

or

- none

3b. How much time in total did you usually spend walking on one of those days?

_____ hours _____ minutes

The last question is about the time you spent **sitting** on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

4. During the last 7 days, how much time in total did you usually spend *sitting* on a **week day**?

_____ hours _____ minutes

This is the end of questionnaire, thank you for participating.

This is the final SHORT LAST 7 DAYS SELF -ADMINISTERED version of IPAQ from the 2000/01 Reliability and Validity Study. Completed May 2001.

17 Appendix H. Participant information sheet 3



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622306)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, 01970 628681)

Dr. Alex Gonzalez de Agüero (alg28@aber.ac.uk, 01970 628560)

Relationship Between Functional Muscular Strength and Bone Mineral Density in Adult Women

Participant Information Sheet

Please Note:

You should inform the investigator if you are pregnant or if there is a possibility that you may be pregnant before you undergo the tests.

Overview

You will only be required to make one visit to the Sport and Exercise Science department. Several measures will be taken during this visit; you will be asked to complete several questionnaires on sunlight exposure, physical activity level, use of oral contraceptives, use of hormone replacement therapy, and menstrual history. You will also be asked to complete a 3-day food diary before attending the laboratory. Height and weight will be measured and recorded, following which a bone and body fat very low-dose x-ray scan and a maximal effort leg strength test will be performed.

Details

The 3-day food diary involves you recording your dietary intake for 3 days before visiting the laboratory. You will be asked to record the type of food you eat (e.g. wholemeal bread), the amount (e.g. 2 slices), and the way it was (e.g. toasted). You will be asked to bring the food diary when you come into the department. If you don't complete the diary before coming into the department, you will be asked to fill in a diary for the previous 3 days.

The scan procedure will involve lying still on a slow moving bed, while tiny quantities of x-rays (in total less than equivalent to 1 chest x-ray) are passed through the body. 3 scans will be performed; a whole body scan, hip scan and a lower spine scan. You will be asked to remove any jewellery and metal (e.g. belts, zips and under wired bras) during the test, as metal will affect the measurement; if there is anything that you cannot remove it may still be possible to take part, but please let the investigator know. You are also asked to let

the investigator know of any metal implants that you have in your body (i.e. pins in bones). It may still be possible to take part in the study, depending on which bones have pins in them. You should also tell the investigator if you are pregnant or if there is a chance you may be pregnant before the scans begin. As well as the scans you will be asked to complete a quick medical questionnaire about your ability to have a scan and to monitor your annual radiation dose.

The procedure for the leg strength test will involve you being securely strapped into a seat, with your knee being held at a 90° angle. To begin with, the natural range of movement of your knee will be measured. You will then be asked to straighten and bend your knee as forcefully as possible; 5 times at a slow speed and then 5 times at a fast speed. Maximum power will be recorded for each speed. Before the test begins you will be asked to complete a short questionnaire about previous injury and illness which could affect your ability to complete a maximum strength test.

Results

The results from your tests will be given and explained to you once all the tests have been completed. You will also then have the opportunity to ask any questions you may have about your results. If any results are found which are not what we expect, your results will be sent to Dr. Phil Jones at Bronglais Hospital, who will then contact you if he thinks it necessary.

Time Commitment

You will only be asked to make one visit to the Sport and Exercise Science Department. It is expected that completing the 3-day food diary before coming into the department will take you approximately half an hour across the 3 days. The visit to the department will last no more than 2 hours.

Your visit to the department will be arranged around your menstrual cycle (where relevant). If you have not yet gone through the menopause and are not reliably on a form of hormonal contraception, you will only be able to take part in the study during the first 14 days after the start of your menstrual cycle (defined as the first day of bleeding).

Benefits

As well as giving useful information on your body composition (body fat mass, muscle mass, and percent body fat) the Dual Energy X-Ray Absorptiometry (DXA) scan used provides feedback on the density of your bones, giving an indication of fracture and osteoporosis risk.

Risks

There are risks associated with radiation and x-rays. However, these risks are minimal due to use of very low-dose scans; the scans used in the current study result in an amount of radiation exposure equivalent to less than 1 chest x-ray. Your radiation exposure will be monitored to make sure that you are within a safe range. There is also a risk of strain or injury associated with the maximal strength production. These risks will be minimised by assessing your natural range of movement and ensuring that movement outside this range is not allowed. You will not be allowed to take part if there is any previous injury or weakness which could make injury more likely.

Confidentiality

All information provided or gathered will be treated with complete confidentiality, and no information that could lead to your identity will be revealed in any reports on the project, or to any other party. If any results are found which are outside normal values, you will be referred to Dr. Phil Jones at Bronglais Hospital. All data collected will be stored securely for an indefinite period of time with a minimum of 6 years for use in research publications; however any data published will be kept anonymous. The data collected may also be used in further research projects and/or by other researchers which have ethical approval.

Complaints and Withdrawal

You have the right to withdraw from the study at any time until publication of the results. You also have the right to withdraw at any time during testing. You have the right to withdraw without giving reason, without prejudice to access of services that are already being provided or may subsequently be provided to you. If you would like to withdraw at any time simply inform Jo Worthington (contact details above). If you are unhappy with any part of your participation you can complain about the study. To complain about the study, you need to write to: Dr. Sarah Riley, Chair of Institute of Human Sciences Ethics Committee, Penbryn 5, Aberystwyth University.

18 Appendix I. Informed consent form 3



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622306)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, 01970 628681)

Dr. Alex Gonzalez de Agüero (alg28@aber.ac.uk, 01970 628560)

Relationship Between Functional Muscular Strength and Bone Mineral Density in Adult Women

Informed Consent Form

I have read and understood the information sheet. The experimenter has fully explained the purpose of the experiment and the possible risks involved. I understand that if there are any results found outside the normal range of values I will be referred to the relevant medical practitioner. I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, I can choose to stop at any time, and that I can withdraw at any stage of the project until publication of the results without being penalised or disadvantaged in any way. I will adhere to the instructions of the experimenter regarding safety before, during and after experimentation.

	Initial Showing Consent
International Physical Activity Questionnaire	
Menstrual Health Questionnaire	
3 Day Food Diary	
Sunlight Exposure Questionnaire	
DXA Scans	
Biodex	

Contact Dr. Jones

I,.....

(Participant's full name)*

hereby volunteer to participate in experimental work as a participant.

Signed

Date

(Participant)

I,

(Investigator's full name)*

certify that the details of this procedure have been fully explained and described in writing to the subject named above.

Signed

Date

(Investigator)

19 Appendix J. Participant information sheet 4



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622619)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, 01970 628681)

Dr. Rhys Thatcher (ryt@aber.ac.uk, 01970 628630)

Dr. Alex Gonzalez de Aguero (alg28@aber.ac.uk, 01970 628560)

Effects of Creatine Monohydrate on Bone Density and on Markers of Bone Turnover in Adult Women

Participant Information Sheet

As well as giving useful information on your body composition (body fat mass, muscle mass, and percent body fat) the DXA scan provides feedback on the density of your bones, giving an indication of fracture and osteoporosis risk.

The current study is an 18-month study, involving low-dose consumption of 3g of a nutritional supplement daily for the course of the study. The supplement will be either a creatine monohydrate supplement or a dextrose placebo. Neither creatine monohydrate nor dextrose has been found to have any adverse side-effects.

The initial visit to the Department will involve anthropometric measures (height and body mass) being taken, followed by DXA scan, a blood sample and a urine sample. Participants will also be asked to answer some short questions on diet and physical activity levels. Participants will then be assigned to either the creatine group or to the placebo group, although they will not know which group they are in. 2 weeks after this initial visit, participants will re-visit the lab, where they will have the opportunity to discuss the study.

From then on, monthly visits to the Sport and Exercise Science Department will be required. At these visits, adherence to the supplement dose will be monitored and participants will have the opportunity to discuss their progress through the study. At the 3, 6, 12 and 18 month visits, follow-up measures will be taken; this involves repeats of the DXA scans, blood and urine samples. At the 18 month study participants will be given their results from throughout the study and will be told which supplement they have been taking.

DXA

The DXA procedure will involve lying still on a slow moving bed, while minute quantities of x-rays (equivalent to 10 days radiation exposure from natural background sources or 1 Chest x-ray) are passed through the body. We will perform a whole body scan, a proximal femur (hip) scan and a lumbar (lower spine) scan. The whole body scan lasts less than 2 ½ minutes and the proximal femur and lumbar spine scans last approximately 10 seconds each.

You will be asked to remove any jewellery and metal (e.g. belts, zips and under wired bras) during the test, as metal will affect the measurement; if these are not removable it may still be possible to participate, but please inform the investigator. You are also asked to inform the tester of any metal implants that you have in your body (i.e. pins in bones). You should also inform the investigator if you are pregnant or if there is a chance you may be pregnant before you undergo the tests. In addition to the scans you will be asked to complete a quick medical criteria questionnaire regarding your capacity to undertake a DXA scan and to ensure that you are well within the legal recommendations for annual radiation doses. Height and weight will also be measured.

Blood Sampling

All blood samples will be taken by individuals fully trained in phlebotomy according to NHS guidelines. The blood sampling will involve sitting still while a tourniquet is loosely applied around the top of the arm. The sample area will be cleaned with an alcohol wipe, and a 5ml blood sample will be taken from the medial cubital vein (the inner elbow). The sample will be separated and the red blood cells, the DNA-containing part, will be removed from the sample. The remaining serum will then be frozen and stored, and all samples will be analysed at the end of the study.

Confidentiality

All information provided or obtained will be treated with utmost confidentiality, and no information that could lead to your identity will be disclosed in any reports on the project, or to any other party. No human DNA will be stored. If any results are found which are outside normal values, you will be referred to the relevant medical practitioner. All data collected will be stored securely for an indefinite period of time with a minimum of 6 years for use in research publications; however any data published will be kept anonymous. The data collected may also be used in further research projects and/or by other researcher which have ethical approval.

All participants have the right to withdraw from the study at any time without prejudice to access of services that are already being provided or may subsequently be provided to the participant. If you are unhappy with any part of your participation you can complain about the study. To complain about the study, you need to write to: The secretary of the Aberystwyth University Ethics Committee for Research Procedures, Deans' Office, Cledwyn Building, Aberystwyth University.

Please Note:

You should inform the investigator if you are pregnant or if there is a possibility that you may be pregnant before you undergo the tests.

20 Appendix K. Informed consent form 4



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622619)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, 01970 628681)

Dr. Rhys Thatcher (ryt@aber.ac.uk, 01970 628630)

Dr. Alex Gonzalez de Agüero (alg28@aber.ac.uk, 01970 628560)

The Effect of a Long-Term Creatine Monohydrate Supplement on Bone Density and Markers of Bone Turnover in Adult Women

Informed Consent Form

I have read and understood the information sheet and have completed the medical criteria questionnaire. The information provided on the questionnaire is correct to the best of my knowledge and I understand it will be treated in the strictest confidence. The experimenter has fully explained the purpose of the experiment and the possible risks involved. I understand that if there are any results found outside the normal range of values I will be referred to the relevant medical practitioner. I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, and that I can withdraw at any stage of the project without being penalised or disadvantaged in any way. I will adhere to the instructions of the experimenter regarding safety before, during and after experimentation.

I,.....

(Participant's full name)*

hereby volunteer to participate in experimental work as a participant.

Signed Date

(Participant)

I,

(Investigator's full name)*

certify that the details of this procedure have been fully explained and described in writing to the subject named above.

Signed

Date

(Investigator)

21 Appendix L. Participant information sheet 5



Institute of Biological, Environmental, and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622078, 07517 235169)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, 01970 628681)

Dr. Alex Gonzalez de Agüero (alg28@aber.ac.uk, 01970 628560)

The Combined Effects of Creatine Monohydrate Supplementation and Exercise on Bone Mineral Density in Adult Women

Participant Information Sheet

The current study is a 12-week study, involving low-dose consumption of 3g of a nutritional supplement daily for the course of the study. The supplement will be either a creatine monohydrate supplement or a micro-crystalline cellulose placebo. Neither creatine monohydrate nor micro-crystalline cellulose has been found to have any adverse side-effects. Participants will be randomly allocated to either an exercise group or to a control group; those in the exercise group will be asked to attend the Department for exercise twice per week for the duration of the study. Those in the control group will be asked to maintain their normal lifestyle.

The initial visit to the Department will involve anthropometric measures (height and body mass) being taken, followed by DXA scan, a blood sample and a leg strength test. Participants will then be assigned to either the creatine group or to the placebo group, although they will not know which group they are in, and will be assigned to either the exercise group or the control group.

From then on, participants in the exercise group will be asked to visit the Department twice per week for the duration of the study in order to take part in the exercise program.

All participants will be asked to visit the Department once every four weeks, at which adherence to the supplement dose will be monitored and participants will have the opportunity to discuss their progress through the study. At the end of the study, follow-up measures will be taken; this involves repeats of the DXA scans, blood sample, and leg strength test. At the final visit study participants will be given their results from throughout the study and will be told which supplement they have been taking.

The DXA procedure will involve lying still on a slow moving bed, while minute quantities of x-rays (equivalent to 10 days radiation exposure from natural background sources or 1 Chest x-ray) are passed through the body. We will perform a whole body scan, a proximal

femur (hip) scan and a lumbar (lower spine) scan. The whole body scan lasts less than 2 ½ minutes and the proximal femur and lumbar spine scans last approximately 10 seconds each. You will be asked to remove any jewellery and metal (e.g. belts, zips and under wired bras) during the test, as metal will affect the measurement; if these are not removable it may still be possible to participate, but please inform the investigator. You are also asked to inform the tester of any metal implants that you have in your body (i.e. pins in bones). You should also inform the investigator if you are pregnant or if there is a chance you may be pregnant before you undergo the tests. In addition to the scans you will be asked to complete a quick medical criteria questionnaire regarding your capacity to undertake a DXA scan and to ensure that you are well within the legal recommendations for annual radiation doses. Height and weight will also be measured.

The procedure for the leg strength test will involve you being securely strapped into a seat, with your knee being held at a 90° angle. To begin with, the natural range of movement of your knee will be measured. You will then be asked to straighten and bend your knee as forcefully as possible; 5 times at a slow speed and then 5 times at a fast speed. Maximum power will be recorded for each speed. Before the test begins you will be asked to complete a short questionnaire about previous injury and illness which could affect your ability to complete a maximum strength test.

All blood samples will be taken by individuals fully trained in phlebotomy according to NHS guidelines. The blood sampling will involve sitting still while a tourniquet is loosely applied around the top of the arm. The sample area will be cleaned with an alcohol wipe, and a 5ml blood sample will be taken from the medial cubital vein (the inner elbow). The sample will be separated and the red blood cells, the DNA-containing part, will be removed from the sample. The remaining serum will then be frozen and stored, and all samples will be analysed at the end of the study.

Participants in the exercise group will be asked to attend the Department twice per week on non-consecutive days for 12 weeks. The exercise session will take the form of a circuit session, with 12 different exercises being used. There will be a mixture of seated and standing exercises, and a mixture of upper and lower body exercises. There will be some progression in the number of repetitions of each exercise throughout the 12 weeks, although the sessions are designed not to be exhaustive.

Benefits

As well as giving useful information on your body composition (body fat mass, muscle mass, and percent body fat) the Dual Energy X-Ray Absorptiometry (DXA) scan used provides feedback on the density of your bones, giving an indication of fracture and osteoporosis risk. Participants in the exercise group will also have the opportunity to engage in a 12-week exercise program free of charge.

Risks

There are risks associated with radiation and x-rays. However, these risks are minimal due to use of very low-dose scans; the scans used in the current study result in an amount of radiation exposure equivalent to less than 1 chest x-ray. Your radiation exposure will be

monitored to make sure that you are within a safe range. There is also a risk of strain or injury associated with the maximal strength production. These risks will be minimised by assessing your natural range of movement and ensuring that movement outside this range is not allowed. You will not be allowed to take part if there is any previous injury or weakness which could make injury more likely. There is a risk of strain or injury associated with the exercise program. These risks will be minimised by the use of gentle, non-exhaustive exercises and ensuring that participants warm up and cool down thoroughly. Participants will be asked to cease exercise if they feel discomfort or pain at any point.

Confidentiality

All information provided or gathered will be treated with complete confidentiality, and no information that could lead to your identity will be revealed in any reports on the project, or to any other party. If any results are found which are outside normal values, you will be referred to a specialist at Bronglais Hospital. All data collected will be stored securely for an indefinite period of time with a minimum of 6 years for use in research publications; however any data published will be kept anonymous. The data collected may also be used in further research projects and/or by other researchers which have ethical approval.

Complaints and Withdrawal

You have the right to withdraw from the study at any time until publication of the results. You also have the right to withdraw at any time during testing. You have the right to withdraw without giving reason, without prejudice to access of services that are already being provided or may subsequently be provided to you. If you would like to withdraw at any time simply inform Jo Worthington (contact details above). If you are unhappy with any part of your participation you can complain about the study.

22 Appendix M. Informed consent 5



Institute of Biological, Environmental and Rural Sciences

Principle Investigator: Joanna Worthington (jpw9@aber.ac.uk, 01970 622078)

Supervisors: Dr. Joanne Wallace (jyw@aber.ac.uk, **01970 628681**)

Dr. Alex Gonzalez de Agüero (alg28@aber.ac.uk, **01970 628560**)

The Combined Effects of Creatine Monohydrate Supplementation and Exercise on Bone Mineral Density in Adult Women

Informed Consent Form

I have read and understood the information sheet and have completed the medical criteria questionnaire. The experimenter has fully explained the purpose of the experiment and the possible risks involved. I understand that if there are any results found outside the normal range of values I will be referred to the relevant medical practitioner. I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, I can choose to stop at any time, and that I can withdraw at any stage of the project until publication of the results without being penalised or disadvantaged in any way. I will adhere to the instructions of the experimenter regarding safety before, during and after experimentation.

	Initial Showing Consent
International Physical Activity Questionnaire	
3 Day Food Diary	
DXA Scans	
Biodex	
Contact Dr. Jones	
Supplementation 3g/day	
Exercise program (if applicable)	

I,.....
(Participant's full name)*

hereby volunteer to participate in experimental work as a participant.

Signed Date
(Participant)

I,
(Investigator's full name)*

certify that the details of this procedure have been fully explained and described in writing to the subject named above.

Signed Date
(Investigator)

23 Appendix N. iPAQ (long)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000).

Assessment of Physical Activity: An International Perspective. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

6. Do you currently have a job or do any unpaid work outside your home?

Yes ☐

No 

Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

No vigorous ☐ job-related physical activity



Skip to question 4

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ hours per day

_____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ days per week

No moderate job-related physical activity



Skip to question 6

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ hours per day

_____ minutes per day

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ days per week

No job-related walking ☐



Skip to PART 2: TRANSPORTATION

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ hours per day

_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ days per week

No traveling ☐ in a motor vehicle



Skip to question 10

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ hours per day

_____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

No bicycling from place to place



Skip to question 12

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ hours per day

_____ minutes per day

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

☐ No walking from place to place

☐ Skip to PART 3: HOUSEWORK,
HOUSE MAINTENANCE, AND
CARING FOR FAMILY

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ hours per day

_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ days per week

No vigorous ☐ activity in garden or yard



Skip to question 16

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ hours per day

_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ days per week

No moderate activity in garden or yard



Skip to question 18

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ hours per day

_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ days per week

☐

No moderate activity inside home



Skip to PART 4: RECREATION,
SPORT AND LEISURE-TIME
PHYSICAL ACTIVITY

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ hours per day

_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ days per week

No walking ☐ in leisure time



Skip to question 22

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ hours per day

_____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ days per week

No vigorous ☐ activity in leisure time



Skip to question 24

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ hours per day

_____ minutes per day

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ days per week

☐

No moderate activity in leisure time



Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ hours per day

_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ hours per day

_____ minutes per day

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ hours per day

_____ minutes per day

This is the end of the questionnaire, thank you for participating.

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

24 Appendix O. Exercises used in sessions

Every session included a 5 minute warm-up and a 5 minute cool-down and stretching period. 12 of the following exercises were used in each session, with equal balance between high-impact and resistance exercises in each session.

- Straight leg lift
- Tricep dips
- Leg extension
- Sit and reach
- Bicep curls
- Mini squats
- Sitting turn
- Standing jumps
- Leg lifts
- Calf raises
- Press-ups
- Sit-ups
- Jumping jacks
- Skipping
- Stationary cycling
- Stationary running
- Step-ups
- Crossbody tricep extensions using exercise bands.